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# COMPARATIVE STUDIES ON THE STRUCTURE OF THE SHOOT APEX IN SEED PLANTS[1]

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(WITH FOUR FIGURES)

## INTRODUCTION

In common with other vascular plants, the typical sporophyte of the seed plants has the ability to produce, throughout its growing periods, a succession of organs and tissues on a theoretically unlimited scheme. This method of development is possible first of all because small areas of dividing and enlarging cells, localized at the apices of shoots and roots, are permanently maintained in a state of potential activity. From these embryonic areas, which are designated as terminal meristems, there arise the primordia of foliar organs and the "primary tissues" of the stem and root.

Following the classical researches on apical growth made by Nägeli during the middle of the last century, much labor was devoted to the study and interpretation of structure and growth in terminal meristems. In particular, the meristematic apex of the shoot, because of its dual role as the initiator of appendages and the primary tissues of the axis, attracted considerable attention. Among the celebrated botanists who contributed to a knowledge of the shoot apex may be mentioned Hofmeister (1857), Hanstein (1868), Pringsheim (1869), Strasburger (1872), Sachs (1878), Haberlandt (1880), and Schwendener (1879, 1885). It is not my intention to review at this time these early pioneering studies, since the details are adequately presented in Schüepp's (1926) monograph on meristems as well as in a recent historical résumé by the writer (Foster 1939b). It is necessary, however, to emphasize that the structural picture of the apex which emerged from most of these studies was that of a complex network of cell walls (cf. especially Sachs 1878). This highly formalized and "lifeless" representation of the cellular structure of the apical meristem resulted in the first place from the crude microtechnique then in vogue, which consisted in the treatment of

[1] Invitation paper read before Section G of the A.A.A.S. at the Philadelphia Meeting on December 28, 1940. It is a pleasure to thank my wife for her assistance in preparing the drawings made to illustrate this paper.



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hand sections with Eau de Javelle or KOH in order to remove completely all protoplasm. But aside from the limitations imposed by technique, great importance was attached to the details of the cell-wall mosaic because of efforts to determine the exact origin of cells either from a single definitive apical cell or from a series of initials. From this standpoint, one of the chief objectives of inquiry in the study of the shoot apex was the plan of cell arrangement with reference to the initials. This could be vividly described and depicted in terms of an intersecting network of cell walls.

The unfortunate result of this restricted form of inquiry was that the larger questions of the cellular organization and activity in the apex as a whole and the nature of its relation to growth and differentiation in the shoot continued to be neglected. Hanstein's (1868) histogen theory, it is true, seemed at first to provide a solution to the problem of tissue differentiation in the stem and root. But the careful studies of Koch (1891, 1893) and Schoute (1903) among others, during the latter part of the 19th century, demonstrated the impossibility of recognizing a rigid correspondence between specific cell layers in the apex and the origin of leaves and the primary tissue systems of the shoot axis (cf. also Foster 1939b). This idea has been further supported by an intensive study within recent years of apical ontogeny in the shoots of certain periclinal chimaeras (Lange 1927). It seems clear from this work that, with respect to the number of generative layers involved, there is no uniform scheme of bud and leaf initiation in the angiosperms investigated and that the problems of tissue and organ determination are not to be solved by a theory of specific histogens (Foster 1936, Jones 1937). Indeed F. O. Bower (1935, p. 328), whose early studies on the terminal meristems of ferns are a classic in morphology, has reached the conclusion that "on the one hand apical segmentation, and on the other morphological definition, whether external or internal are distinct processes, each of which is determined by the apical region as a whole, and not by its segments."

The above quotation from Bower serves to show the unsatisfactory state of our knowledge of the role of the shoot apex in the differentiation process. We must admit, I think, that the continued emphasis merely on the formal questions of cell origin and cell arrangement in apical meristems holds little or no promise of furnishing additional insight into this fundamental problem. What is needed is a more realistic approach, utilizing the advantages of modern botanical technique in an effort to study all possible aspects of cell structure and cell activity rather than simply the mosaic of cell walls.

The purpose of this paper is to describe the most significant results of certain very recent cyto-histological studies on the shoot apex of seed plants. One of the most interesting aspects of these exploratory studies is the demonstration that the so-called "primordial meristem" of the apex varies con-

siderably in its structure in different plants and that it should no longer be regarded simply as an "undifferentiated" or homogeneous tissue as is done by such authors as Haberlandt (1914, p. 73). On the contrary, the apical meristem is segregated into more or less well-defined *tissue zones*. These zones are distinguished from each other by such features as cell size, nuclear size, planes of cell division, relative frequency of mitosis, reaction to plasma and nuclear stains, and relative thickness of cell walls. As I shall show in describing the various types of zonal patterns, this structural diversity in the so-called primordial meristem is not only interesting from the standpoint of comparative anatomy but also appears to throw new and important light upon the nature of organ and tissue differentiation. For convenience and emphasis, the observations will be separately described under the main groups of gymnosperms and angiosperms which have been examined from the new point of approach.

## GYMNOSPERMS

Recent investigations have revealed a number of interesting types of zonal structure in the shoot apex of the gymnosperms. Since the limitations of space do not permit of a detailed contrast between the various types of zonal patterns, I shall attempt to summarize only the most significant of the new facts.

*Coniferales.* The apex of *Abies venusta* (Dougl.) K. Koch illustrates a zonal pattern which is apparently wide-spread in the conifers (Koch 1891, Korody 1937, Cross 1939, Foster 1939a). In this type, the tissue of the apex is segregated into two well-defined zones, viz.: an outer *peripheral zone* of deeply-stained, actively-dividing cells from which ultimately arise the leaf primordia as well as the epidermis, cortex, and provascular tissue; and an inner core or *central-tissue zone*, composed of filamentous groups of highly-vacuolate elongating and dividing cells, which is the precursor of the pith (fig. 1). Both of these zones originate from a small ill-defined subterminal group of "mother cells" which in turn have arisen from the inner derivatives of the apical initials (fig. 1a). Except for a more regular surface cell layer, this zonal pattern is duplicated fundamentally in the apex of *Araucaria Bidwilli* Hook., and Cross (1939) has recently described a similar type of zonal structure for the apical meristem of the shoot of *Taxodium distichum* (L.) Rich. One of the most significant features of this type of apical structure is the inequality between the two zones with respect to the *relative rates* of cell division, cell enlargement, and cell maturation. The *central tissue zone* is typified by the polarized growth and the rapid progressive elongation of the cells from their point of origin. In contrast, the *peripheral zone* exhibits less evidence of early specialization and appears to consist of a rather uniform tissue in which cell division rather than cell enlargement predominates. Further consideration of the implication of these differences will be deferred until other types of zonal structure have been described.

*Ginkgoales and Cycadales.* As representatives of the two most primitive groups of living seed-bearing plants, *Ginkgo* and the cycads have naturally attracted much morphological study and speculation. But it is only within the last two years that information has appeared regarding the cyto-histology of the shoot apex of these archaic seed plants. A priori, it might be assumed that the so-called "primordial meristem" of the apices of these ancient spermatophytes would be simple and undifferentiated in structure. On the contrary, the apical meristem exhibits, in a very impressive way, a distinct and complex type of zonation.

In *Ginkgo* (Foster 1938) the apices of both spur and long shoots are fundamentally similar in zonal structure. The most distinctive feature is furnished by the *central mother-cell zone* which consists of a well-demarcated cup-shaped group of large, lightly-stained cells situated *directly* beneath the apical initials (fig. 2). Because of their large size, irregular arrangement, huge nuclei, prominent and unevenly thickened walls, and relatively infrequent divisions, the central mother cells diverge strongly from the features usually associated with the primordial meristem in the seed plants. Of particular interest, however, is the renewed mitotic activity which appears at the lateral and basal margins of the zone of central mother cells, since from these areas there originate the bulk of the *peripheral tissue zone* and the *zone of rib meristem* (fig. 2b). The relation of this peculiar zonation to the differentiation process is interesting. As in many conifers, the *peripheral tissue zone* is composed of deeply-stained actively dividing cells and ultimately gives rise to the foliar organs, epidermis, cortex, and provascular tissue. But with reference to the origin of the pith, an important difference exists. In *Abies,* for example, this tissue is propagated directly by the progressive basipetal maturation of the cells of the central tissue zone (figs. 1, 1a). In *Ginkgo,* however, there is interpolated between the apical initials and the rib meristem from which the pith originates, a *central mother-cell zone,* distinctive in its structure and characterized by "random" cell expansion, rather than uniform cell division. From the standpoint of growth, this zone therefore may be regarded as a region of pronounced cell enlargement maintained between two well-defined areas of more active cell multiplication (figs. 2a, 2b, 2c).

Our knowledge of the cyto-histology of the shoot apex of cycads is at present unfortunately limited to the results of studies on three genera, so that caution must be exercised in making comparisons. Nevertheless, it does appear significant that a similarity exists between the zonal structure of the apex of *Ginkgo* on the one hand and the species of cycads examined, on the other. The resemblances seem particularly interesting in view of (1) the much greater size of the apex in the cycads, which may reach the enormous diameter of 3.5 mm. in *Cycas revoluta* Thunb., and (2) the striking differ-

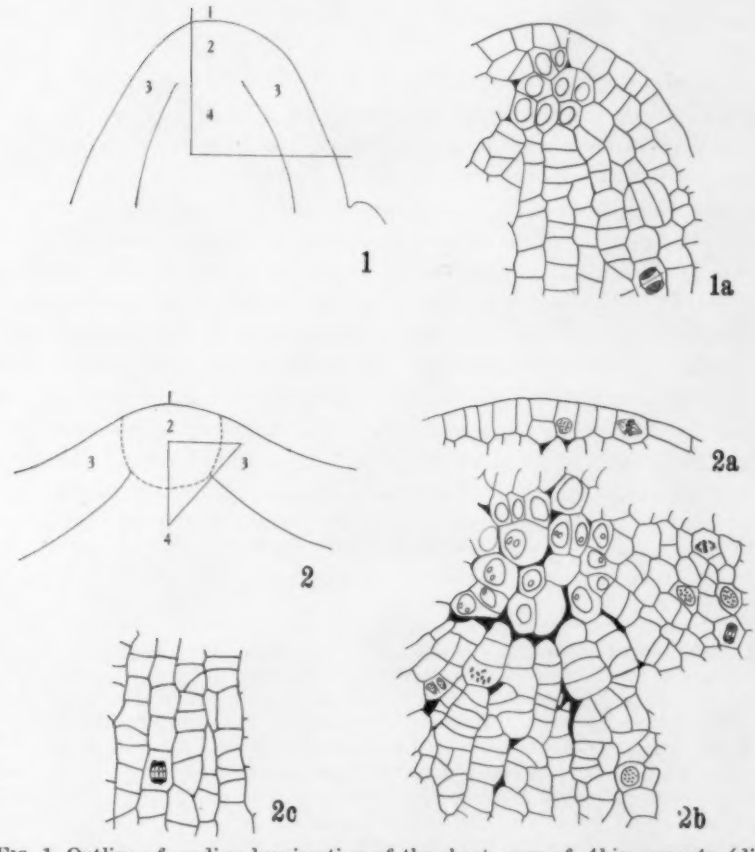



FIG. 1. Outline of median longisection of the shoot apex of *Abies venusta* (diameter at base 230 microns) showing the position and extent of the apical initials (1), the subterminal "mother cells" (2), the zone of peripheral tissue (3) and the central-tissue zone or rib meristem (4). FIG. 1a, cellular structure of the sector of the apex indicated in figure 1. The small group of subterminal mother cells, shown with nuclei, is the common point of origin of rib meristem and peripheral tissue. Further explanation in the text. In this, and all subsequent drawings of cell detail, the magnification is approximately 250 diameters.

FIG. 2. Outline of median longisection of the apex of a long shoot of *Ginkgo biloba* (maximum diameter 321.75 microns) showing the position and relationships of the apical initials (1), the cup-shaped zone of central mother cells (2), the zone of peripheral tissue (3) and the rib meristem (4). FIG. 2a, the surface cells at the summit of the apex. Note prophase and recent periclinal division in the two apical initials shown in this section. FIG. 2b, cellular structure of the triangular sector of the apex indicated in figure 2. The zone of central mother cells is well-defined by the large size, irregular arrangement and shape, huge nuclei and uneven wall-thickenings of its component cells. The point of origin of the rib meristem and peripheral tissue from this zone (represented diagrammatically in figure 2 by the broken outline) is shown by a renewal in mitotic activity and a marked decrease in cell size. Note that certain of the original wall-thickenings of the central mother cells are "carried over" into the young rib meristem. FIG. 2c, small portion of rib meristem below that shown in figure 2b. Observe the filamentous cell-series and the absence of irregular wall-thickenings.

ences between the morphology of the shoot system of cycads and the maidenhair tree.

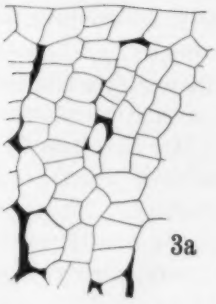
The zonal pattern of the shoot apex of *Dioon edule* Lindl.[2] is remarkably like that of *Ginkgo*. A deeply-situated and clearly delimited *central mother-cell zone*, derived from an extensive series of "initials," is the common point of origin for the massive zone of rib meristem and the bulk of the *peripheral tissue zone* (figs. 3, 3a). As in *Ginkgo*, these two zones can be traced to a renewed intensity in cell division at the edges and base of the zone of central mother cells (figs. 3b, 3c). Apparently a distinctive character of this zone, as contrasted with *Ginkgo*, is the greater variability in the size of the component cells. This probably reflects the wide degree of fluctuation in the relative frequency of cell division and cell enlargement in various portions of this zone. Johnson (1939) has recently examined the zonal structure of the apices of three Florida species of *Zamia*. It is evident from his drawings and descriptions as well as from observations made by the writer (1939a) that the histology of the apical meristem in these forms is closely similar in basic

**Explanation of figures 3 and 4**

FIG. 3. Outline of median longisection of the shoot apex of *Dioon edule* (maximum diameter 1677 microns) showing the position and relationships of the extensive zone of initiation (1), the deeply-situated zone of central mother cells (2), the peripheral tissue zone (3) and the broad zone of rib meristem (4). Note the foliar primordia at the base of the dome-shaped apex. FIG. 3a, cellular structure of small sector through the initiation zone, showing a periclinally divided surface cell and the tiered arrangement of the inner cells. Note the irregular wall thickenings. FIG. 3b, cellular structure of the triangular sector of the apex indicated in figure 3. As in *Ginkgo* (fig. 2b) the addition of new cells to the zones of rib meristem and peripheral tissue occurs respectively at the base and sides of the zone of central mother cells (broken outline in figure 3). Note the wide variation in size and form of the central mother cells (shown with nuclei) and the massive wall-thickenings. These decrease markedly in prominence in the distal portions of zones 3 and 4. FIG. 3c, small portion of the rib meristem below the region shown in figure 3b. Prominent wall-thickenings are absent, as in the comparable region of the apex of *Ginkgo* (fig. 2c).

FIG. 4. Outline of median longisection of the very broad shoot apex of *Cycas revoluta* (maximum diameter 3412.5 microns) showing the four main tissue zones (zones 1–4). In contrast to *Ginkgo* and *Dioon*, there is no sharp demarcation between the central mother cells (2) and the adjacent zone of rib meristem (4). The broken outline however indicates that most of the peripheral tissue zone (3) originates from the edge of zone 2. The outer portion of the peripheral tissue, shown by the double outline on the flanks of the shoot apex, consists of the surface cells and their recent periclinal derivatives. FIG. 4a, cellular detail of small sector through the initiation zone (zone 1, fig. 4) showing frequency of periclinal divisions in the surface cells. FIG. 4b, a small group of central mother cells. Note large size of cells and prominent wall-thickenings. FIG. 4c, cellular structure of a small portion of the transition region between zones 2 and 3, showing the characteristic renewal in mitotic activity and the origin of the obliquely-arranged cell groups of the peripheral tissue. FIG. 4d, details of cell structure and arrangement at the point of origin of the rib meristem from the central mother cells (shown with nuclei).

[2] A detailed account of the structure of the shoot apeex of this cycad will be published in the near future.



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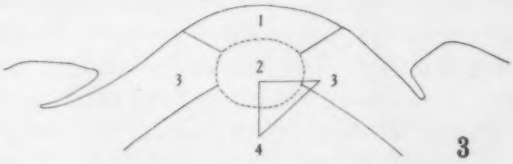


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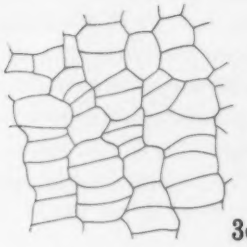

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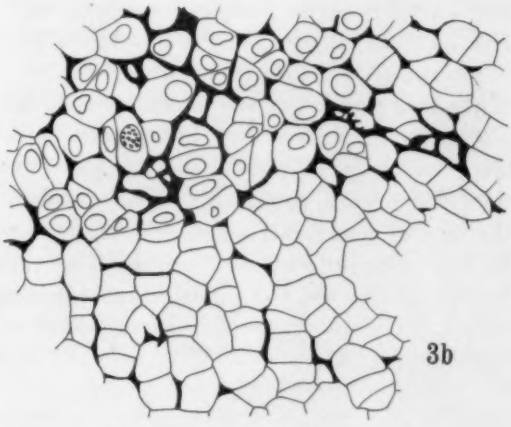

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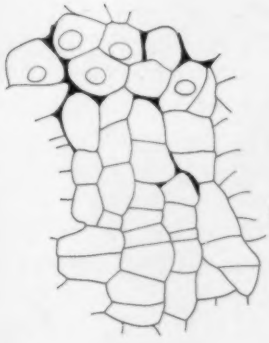

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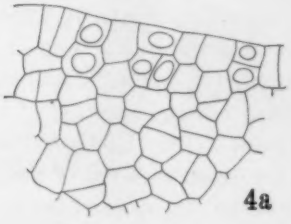

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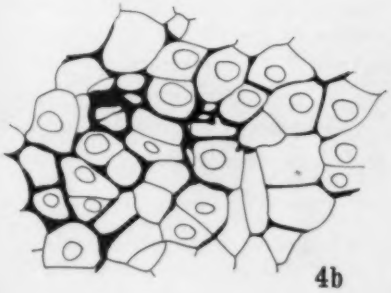

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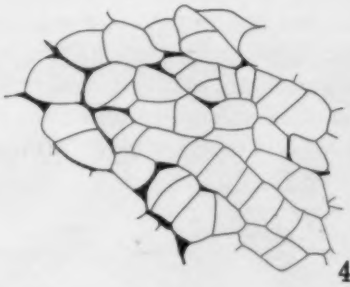

4c

type to that of *Dioon edule*. The most intensive study, however, has been devoted to the shoot apex of *Cycas revoluta* (Foster 1939a, 1940). Whether one examines the relatively delicate conical apex of the seedling or the huge plateau or dome-shaped meristem of the adult plant, it is apparent that the *central mother cell zone* is less sharply demarcated than in either *Ginkgo*, *Dioon*, or *Zamia* (fig. 4). This is chiefly so because there exist no sharp boundaries between pith, rib meristem, and central mother cells (figs. 4b, 4d). In short, the renewal of mitotic activity in *Cycas* is most clearly seen at the edges of the central mother cells in connection with the propagation of the inner region of the zone of peripheral tissue (fig. 4c). All the species of cycads which have been investigated agree with *Ginkgo* in that the relatively enormous pith of the shoot axis originates from the rib meristem while the foliar organs, provascular tissue, epidermis, and cortex are produced by the zone of peripheral tissue. The latter, however, is not uniform in respect to the size or the staining qualities of its cells. Especially in *Cycas revoluta*, it consists of a shallow outer region made up of very small, deeply-stained cells and a more extensive inner region of larger and more obviously vacuolate cells (fig. 4).

### ANGIOSPERMS

The fact is now well-established that in many angiosperms the cells of the shoot apex are arranged in two distinct zones, viz.: an outer zone or *tunica* composed of one or more layers of small, essentially similar, thin-walled cells enclosing an inner zone or *corpus* in which the cells are irregular in size and arrangement.[3] Under the influence of the tunica-corpus theory (Foster 1939b, Rüdiger 1939) the chief points for investigation have been (1) a determination of the number of layers composing the tunica and (2) the respective roles of tunica and corpus in the initiation of leaf and axillary-bud primordia. As a consequence, very little attention has been devoted to the cyto-histology of the shoot apex in flowering plants. It is clear, however, even with the meagre information at our disposal, that in addition to the more familiar type of shoot apex, there exist other types of zonal structure some of which invite direct comparison with the conditions just described for the more primitive gymnosperms. Furthermore, Gregoire (1938) has recently concluded that there is a fundamental difference between the zonal structure and growth of the terminal meristem of the flower and the vegetative shoot in the angiosperms (cf. Foster 1939b). Unfortunately, however, our knowledge of the comparative histology of vegetative and floral apices is at present meagre and final judgment of Gregoire's radical viewpoint must await the results of further research (cf. especially Brooks 1940 and McCoy 1940).

[3] cf. postscript.

*Cactaceae.* Boke[4] has recently completed a study of the structure of the shoot apex in two species of this much-neglected family, viz.: *Opuntia cylindrica* DC. and *Trichocereus spachianus* Riccob. In both of these species, the surface cells of the apex form a more or less discrete layer or "tunica." The internal tissue of the apex, however, does not correspond in structure or behavior to the corpus region of such "typical" angiosperms as *Carya* (Foster 1935), *Rhododendron* (Foster 1937), or *Acacia* (Boke 1940). On the contrary, a zonal pattern obtains which recalls the situation in the apex of *Ginkgo* or *Dioon*. From the sides and base of the well-defined zone of vacuolated, irregularly arranged thick-walled cells there arise respectively the peripheral tissue zone and the zone of rib meristem. The latter differentiates into the pith and in *Trichocereus* also into the medullary vascular system while the peripheral tissue zone gives rise to the leaves, areoles, epidermis, cortex, and the main system of vascular bundles.

*Palmaceae.* Ball[5] has made a comparative study of the shoot apices of *Phoenix canariensis* Chaub., *Phoenix dactylifera* L., *Washingtonia filifera* Wats., and *Trachycarpus excelsa* Wendl., and the results of this investigation clearly indicate the existence of two distinct types of zonal structure. In *Washingtonia* and *Trachycarpus*, the tunica is biseriate and lies directly in contact with a poorly-defined corpus zone from which the rib meristem originates. The apex of *Phoenix*, however, exhibits a very divergent zonation. This consists in the presence of a large zone of small, lightly-stained, and irregularly-arranged cells from the sides and base of which, by means of cambial-like cell divisions, there arise respectively the peripheral and rib meristem zones. A further but less significant contrast is produced by the presence in *Phoenix* of a uniseriate tunica.

## SUMMARY

A brief description and comparison have been given of the main results of recent studies on the cyto-histology of the shoot apex in certain gymnosperms and angiosperms. The most significant implications of these investigations may be summarized as follows:

1. The so-called primordial meristem of the shoot apex in seed plants cannot be regarded as either "simple" or "undifferentiated" in structure. On the contrary, the cells of this meristem are segregated into more or less well-defined *tissue zones*, which are demarcated by such characters as cell size, the structure and intensity of staining of the protoplast, the relative thickness of the cell wall, and the proportional rates of cell division and cell enlargement.

2. The extent and relationship between the various tissue zones appear to reflect the type, direction, and distribution of growth in the apex. From

[4] The results of this study will be published shortly.

[5] A detailed paper describing this investigation is in course of preparation.

this aspect, the various zonal patterns which have been described are of interest from a morphogenetic as well as a purely comparative standpoint. In the *peripheral tissue zone*, growth is manifested largely in cell division and there is a pronounced tendency to delay cell maturation. It seems significant therefore that this highly active zone is the area of initiation of foliar structures and provascular tissue in various groups of seed plants. The *zone of central mother cells*, which is so prominent in the apices of some plants, is a region characterized by impressive *cell enlargement* rather than "uniform" cell division (figs. 2b, 3b, 4b). The renewed mitotic activity which occurs at the sides and base of this zone is very suggestive of the accumulation of some type of hormone which stimulates or controls cell division. Finally, the *zone of rib meristem* which is composed of vertical, filamentous series of genetically-related cells, represents a tissue of the apex typified by polarized growth and progressive enlargement and cell maturation (figs. 1a, 2c, 3c, 4d).

3. In the light of the studies reviewed in this paper, it is very doubtful whether the importance usually attached to the "initial" cell or cells in a growing apex is justified. On theoretical grounds, it is admitted that in many gymnosperms all the tissue of the apex originates from the division of surface cells (figs. 1a, 2a, 3a, 4a). But whether these cells are constant in number, form, and sequence of division is highly doubtful. In the massive apex of *Cycas revoluta* for example, it is obvious that a very large number of morphologically identical surface cells and their recent derivatives collectively represent an *initiation zone*. However, no one cell or group of cells seems to dominate in this zone (fig. 4a). Comparable difficulties also arise with the more delicate apices of such angiosperms as *Acacia*. Here the zonal pattern consists of three more or less discrete tunica layers which envelop a central zone or *corpus*. According to Boke's (1940, p. 77) observations, however, "tunica and corpus are not rigidly defined" zones and furthermore it proves impossible to designate any particular series of superposed cells as the "initials" of the tunica layers. In view of these facts, it seems that the question of the number of initial cells is relatively insignificant as compared with the broader problem of the coordinated pattern of growth which expresses itself in the various types of zonal structure which have been described.

4. The picture of the apex which emerges from these studies is essentially dynamic in character. Indeed the evidence from comparative histology supports the recent conclusion of Thoday (1939) that "the shoot apex is a self-determining and dominant center of development."

5. From the standpoint of our present knowledge, there is no sure indication as to the nature of the most primitive type of apical meristem in the shoot of seed plants. The zonal pattern in the apices of *Ginkgo* and certain of the cycads may well represent an extremely ancient condition. But the

striking parallel in zonal structure furnished by certain angiosperms indicates the urgent need for a continued systematic exploration of the cytohistology of the shoot apex of seed plants along broad comparative lines.

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*Postscript.* The tunica-corpus theory has received support from the recent experimental studies of Satina, Blakeslee and Avery (1940a) whose paper was published after the presentation of the present article. These investigators succeeded, by means of treating seeds with dilute solutions of colchicine, in inducing various kinds of periclinal chimeras in *Datura stramonium*. It was found that each of the two layers of the tunica, as well as the outermost "layer" of the corpus of the shoot apex responded *independently* to the colchicine treatment in that "any one of these germ layers in the chimeras may remain diploid, with cells containing 24 chromosomes, while the others become polyploid with tetraploid or octoploid cells." These variations in chromosome number and size of the cells are particularly useful from an histogenetic standpoint because they provide a way of determining accurately the role, in this plant, of tunica and corpus in the initiation of foliar and floral organs and in the differentiation of the primary tissues of the axis (cf. also Satina, Blakeslee and Avery, 1940b). In the writer's opinion, however, the attempt of these authors to apply Hanstein's (1868) terms "dermatogen," "periblem," and "plerome" in the description and interpretation of the structure of the shoot apex in *Datura* is likely to lead to confusion and, from a comparative viewpoint, is quite unjustified. As I have shown in a recent article (Foster 1939b), Hanstein's histogen theory has no general validity for the seed plants and hence its basic assumptions, while applicable to certain *individual* cases, do not assist in understanding the fundamental nature of organ and tissue differentiation at the shoot apex in the spermatophytes as a whole (cf. especially Schoute 1903, pp. 90–93, and Sharman 1940).

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# THE PROLIFERATION OF DANDELIONS FROM ROOTS[1]

E. NAYLOR

(WITH SIXTEEN FIGURES)

Very few scientific investigations have been made on the propagation of dandelions from the roots, although many have observed that new plants appear after the tops have been removed. Munson (1903) says that the general practice of cutting "greens" causes more plants to appear. As many as 6 new crowns were observed on plants which had been decapitated. McCallum (1905) in a series of papers on regeneration in plants merely remarks that when roots of *Taraxacum* are cut into several pieces each piece formed new shoots. Longyear (1918) devotes a small portion of a paper on dandelions to propagation by root cuttings, and says that roots produce new plants at any place when cut. Pieces an inch long formed new plants after 18 days. He also observed that clusters of many new plants appeared after the tops had been removed from old plants growing in a lawn.

In the present study as many as 20 separate shoots have been isolated from the end of a single root cutting 1 cm. in diameter. A small ring of greenish callus develops near the center of the cut surface, and from this tissue the young leaves are differentiated in great profusion. Such remarkable capacity for vegetative reproduction from the roots explains the difficulty of eradicating this common plant.

## METHODS

Cuttings were obtained from a small group of plants growing in an open lawn at the New York Botanical Garden. These plants have been identified as *Taraxacum laevigatum* (Willd.) DC.[2] The roots were washed and cut into transverse segments varying in length from 4 to 6 cm. After removal of the latex from the cut ends with cold water, the root pieces were placed upon moist filter paper in Petri dishes. Transverse and longitudinal sections were removed from the cut surfaces at daily intervals, fixed in medium chromo-acetic acid, dehydrated in ethyl alcohol, and embedded in paraffin. Sections were cut 8 microns in thickness and stained with Heidenhain's iron-alum haematoxylin or with Delafield's haematoxylin and safranin.

A maceration method described by Curtis (1940) was used to aid in the identification of the phloem elements. Small pieces of tissue were allowed to stand 24 hours in a mixture of one part of hydrochloric acid to four parts of 95 per cent ethyl alcohol, then washed in 10 per cent ammonia water. The separated cells were stained with an alcoholic solution of anilin blue.

[1] This study was made at the New York Botanical Garden during a leave of absence granted by the University of Missouri.

[2] Specimens are deposited in the Herbarium of the New York Botanical Garden.

For the study of the primary root seedlings were grown on moist filter paper and in sand cultures. Seeds collected at various times during the summer and fall germinated within two or three days.

## STRUCTURE OF THE ROOT

Several investigators have contributed to our knowledge of the internal structure of the dandelion root. De Bary (1884) described the fleshy root and concluded that it was composed of secondary phloem which consisted of parenchyma, sieve tubes, and articulated laticiferous vessels. Jeffrey (1917) remarked that the phloem was much better developed than the xylem, and contained certain dark concentric bands. These circular bands not only mark the position of the latex tissue, but that of the sieve tubes as well, which cannot be clearly distinguished from the former. Haberlandt (1914) described in some detail the articulated latex tubes of plants, and remarked that they were characteristic of the Cichoriaceae. Curtis (1940) has given the most complete description of the phloem tissues in the dandelion root. By the use of special macerating methods he was able to demonstrate the sieve tubes and latex elements arranged in concentric zones in the parenchyma. The findings here presented are in harmony with those of Curtis.

The primary root and the first leaf are well differentiated in a seedling 10 days old, as shown in figure 1.

A slight thickening immediately below the cotyledons is already evident; it is this region which continues to enlarge to form the fleshy root, as is demonstrated in figure 2.

Internally the primary root exhibits the usual arrangement of epidermis, cortex, and stele. The primary xylem is diarch (fig. 3). The phloem is largely parenchymatous, and the pericycle is often difficult to distinguish. The endodermis consists of a single row of cells with walls not much thicker than those of surrounding tissues. The cells of the cortical parenchyma are large and thin-walled, and some show signs of disintegration and the formation of air spaces. Granular materials present in some of the cortex cells give a protein reaction with nitric acid. Starch is not present in any portion of the root. The epidermis is variable in appearance. It may consist of a single layer of thick-walled cells, or it may have collapsed and partially disappeared so that the outer cells of the cortex form the surface of the root.

In a seedling four weeks old secondary development has started in the xylem and phloem regions, and other changes are evident within the cortex. Numerous scalariform vessels develop on either side of the primary xylem, and many parenchymatous phloem cells are rapidly differentiated. The cells of the endodermis undergo numerous divisions, new radial walls appearing at regular intervals (fig. 4). The pericycle divides both radially and tangentially and keeps pace with the endodermis in the rapid increase in diameter of the stelar region.

The fleshy root of a plant several months old consists almost entirely of secondary tissues. Two major regions can be identified in transverse sections; the relatively small central cylinder of xylem, and many concentric bands of secondary phloem which somewhat resemble annual rings. These concentric bands, which make up the bulk of the root, consist of rather narrow zones of sieve tube and latex cells which alternate regularly with similarly arranged broader zones of parenchymatous cells. The latter cells have thin walls, and occur in regular vertical and radial rows corresponding in arrangement to the cambium cells from which they originate (fig. 8). At fairly regular intervals the parenchyma interrupts the bands of sieve tube and latex cells, forming indistinct phloem rays. It was not found possible to determine the age of the root by counting the bands of secondary phloem.

The central cylinder of xylem is composed of secondary tracheids and tracheae and xylem parenchyma, as shown in figure 6. There is little evidence of ring formation in this region. A single cambium layer is found outside the last formed secondary xylem.

Laticiferous cells are abundant in the dandelion root, confined to the phloem region. These cells appear to originate from the cambium; they elongate rapidly and coalesce to form continuous tubes. The result is a complex net-like formation, very closely associated with the sieve tubes and embedded among the elongated cells of the parenchymatous phloem, as illustrated in figures 5 and 7. The sieve-tubes are long and narrow, not numerous, and have sieve openings only on the end walls. Both the latex and sieve tube cells are difficult to demonstrate in sections, but can easily be observed after macerating and staining.

There is no trace of the pericycle, endodermis, cortex, or epidermis in old fleshy roots. A phellogen layer is found near the periphery of the root, where it originates in the parenchyma cells of the secondary phloem. In young roots the phellogen appears first in the outer cortex, later in the pericycle. Secondary thickening takes place rapidly in the dandelion root and results in the addition of numerous bands of secondary phloem and a small amount of secondary xylem.

## THE DEVELOPMENT OF NEW PLANTS

The root pieces selected for study were mostly from the middle of the fleshy root; they measured from 8 to 10 mm. in diameter. The callus appears on the second or third day in the form of a band lying immediately outside the xylem cylinder and completely surrounding it, as illustrated in figure 14. After five or six days the callus has developed a green color and has organized numerous leaf primordia, as shown in figure 15. The callus enlarges rapidly, and grows out over the cut surface so that one may sometimes make a false assumption about its origin. The young leaves differentiate quickly

and their dentate margins are easily recognizable on the seventh or eighth day; they may reach a length of 2 cm. on the tenth day after the cutting is made (fig. 16).

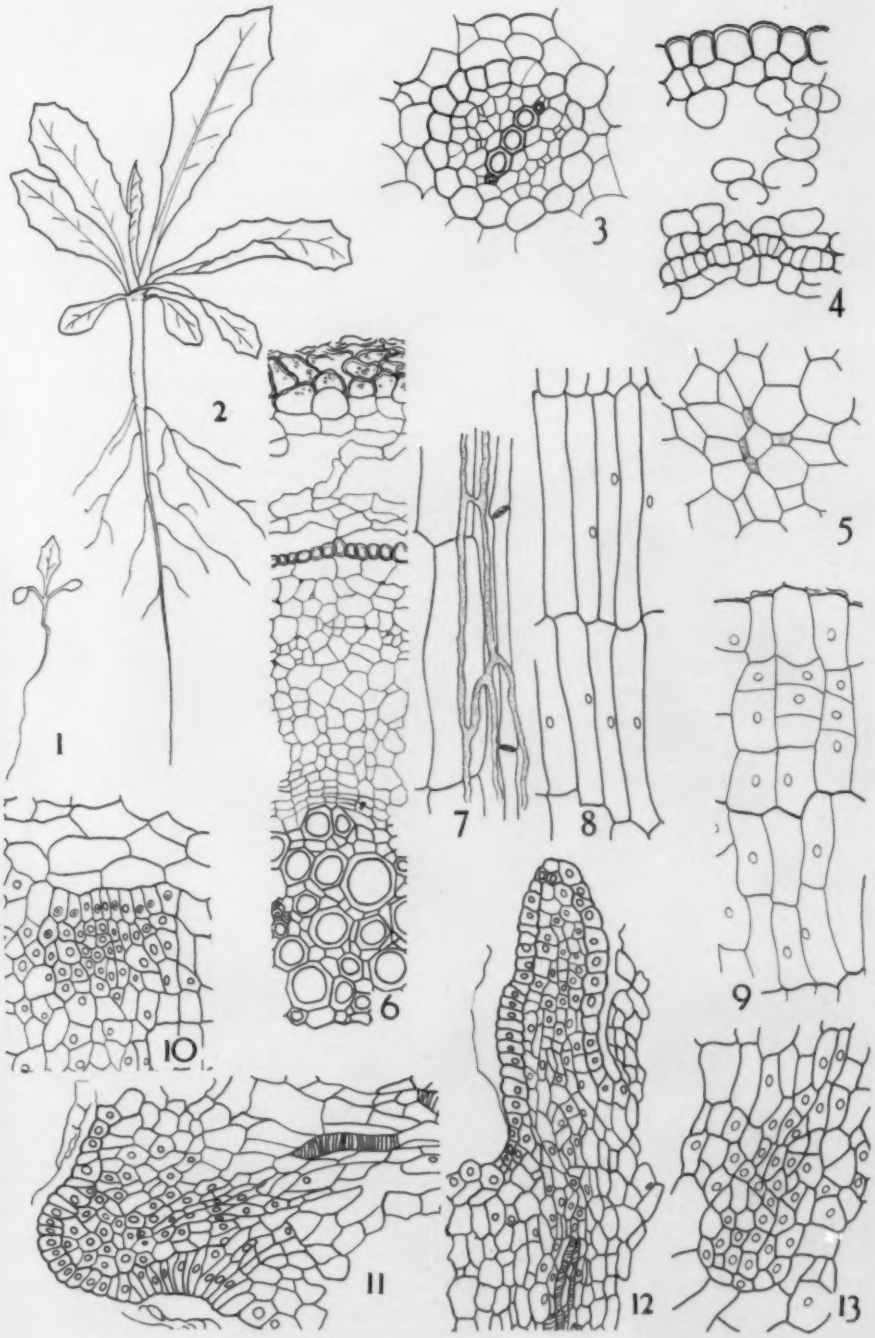
The first internal evidence of new growth appears on the second day in the parenchymatous cells of the last formed two or three bands of secondary phloem near the xylem cylinder. The cells responsible for the new parts are considerably elongated, as shown in figure 8. Mitotic divisions occur at right angles to their long axes. Such activity may be found in the outermost cells of the cut surface, as in the cutting shown in figure 9, or may be confined to cells which are several cell-layers below the wounded surface. Rapid enlargement of these newly formed cells of the phloem parenchyma results in the external appearance of concentric rings of callus. Continued mitotic activity soon renders it impossible to distinguish the original parenchymatous cells; the result is a homogeneous mass of meristematic cells. The outer bands of secondary phloem near the periphery of the root are never active in proliferation.

Differentiation proceeds rapidly and by the third day small groups of cells containing densely staining protoplasm can be identified from one to several cells below the surface (fig. 10). These are the leaf primordia, which at this early stage of development have a distinct cellular pattern. Each consists of a central mass of cells in which divisions occur at various angles, and a single outer layer of cells in which anticlinal divisions predominate. Rapid cell divisions and elongation in the center of the mass cause the entire primordium to assume the shape of an inverted cone, as shown in figures 11 and 14. From these masses of cells the young leaves are differentiated in about 3 days. The details of the development of the leaf blade were not studied.

The development of vascular tissue begins at approximately the same time that the leaf primordia are initiated. Groups of meristematic cells situated deeper within the callus than the leaf primordia produce short vessel

**Explanation of figures 1–13**

FIG. 1. Seedling 10 days old. × 0.4. FIG. 2. Seedling 4 weeks old. × 0.6. FIG. 3. Stelar region of very young root. × 190. FIG. 4. Cortex of young root showing radial divisions in cells of the endodermis, also formation of air spaces. × 190. FIG. 5. Sieve tube cell, latex cells (stippled), and surrounding parenchyma of the secondary phloem. × 200. FIG. 6. Cross section of young root after secondary thickening has begun. Epidermis replaced by outer cortex parenchyma, endodermis still evident, and stele making up major portion of the root. × 180. FIG. 7. Elongated sieve tube cell, branching latex cells, and phloem parenchyma. Preparation made after macerating and staining with anilin blue. × 200. FIG. 8. Phloem parenchyma cells, showing the regular arrangement. × 180. FIG. 9. Cell formation after two days in phloem parenchyma underneath the cut surface. × 200. FIG. 10. Formation of leaf primordium on fourth day. × 180. FIG. 11. Leaf primordium on fifth day beginning to break through surface layers. × 180. FIG. 12. Leaf primordium on seventh day; vascular system partly established. × 180. FIG. 13. Root formation from the distal end of the cutting after two weeks. × 180.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segments, which at first have no particular orientation toward the vascular system of the old root. The vessels are mostly of the scalariform or reticulate type; pitted forms were not found. Differentiation of such xylem elements takes place very rapidly, and the order of maturation is both forward toward the surface of the callus, and in the opposite direction toward the phloem parenchyma. This activity continues until the vascular connection is completed between the leaf primordium and the secondary xylem. The vascular cambium may also form additional xylem cells. Occasionally vascular tissues were found to develop within the callus before the formation of the leaf primordia. The degree of differentiation of new parts is not the same at any one time in the entire callus, as is illustrated in figures 14 and 15. Leaf formation may continue for several weeks.

New roots developed from the distal ends of the cuttings, and generally

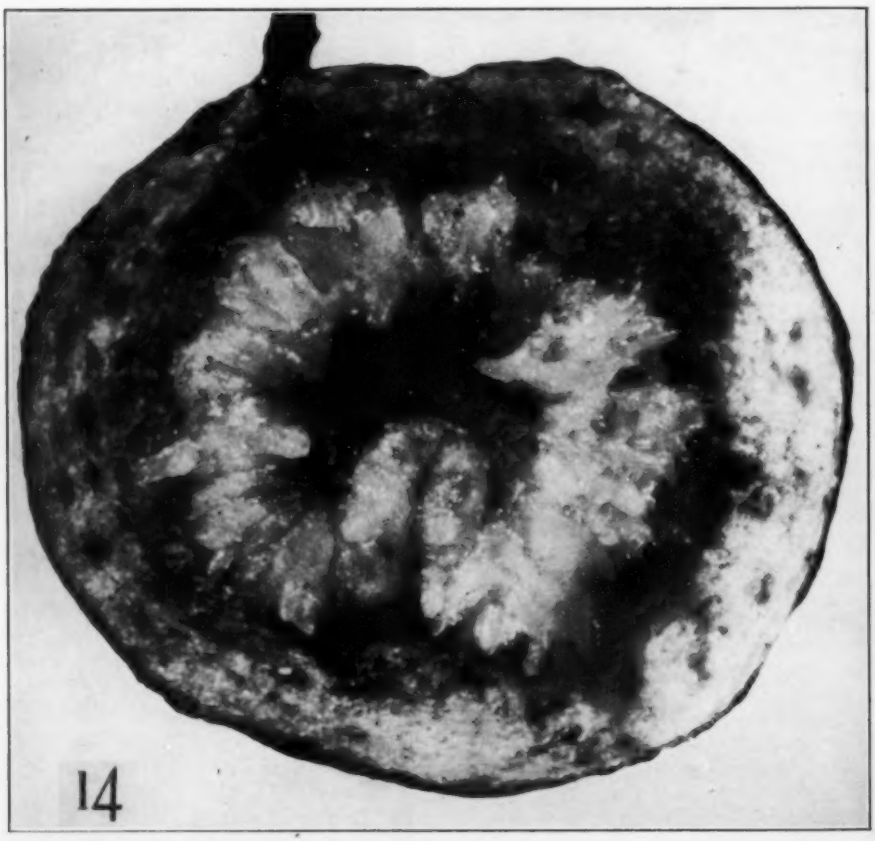


FIG. 14. End view of root, showing development of the ring of callus and leaf primordia after five days. (Photograph by courtesy of the New York Botanical Garden.)

did not appear until several days or even weeks after the shoots had formed at the proximal end. Their origin is very similar to that of the shoots. A band of callus is produced outside the xylem parenchyma, but its growth is not very vigorous, and it remains in a semi-dormant condition while shoot development takes place at the proximal end of the old root. The new roots emerge directly from the callus. A few lateral roots appeared near the stumps of old branch roots which had previously been removed.

Internally the callus at the distal end of the cutting, like that at the proximal end, originates from the phloem parenchyma. The root tips are formed from meristematic cells located a short distance below the surface (fig. 13). Vascular connections are established in a way similar to that described for the shoots. Roots have not been observed to develop with the shoots at the proximal end of root cuttings.

## POLARITY

Leafy shoots were the only new parts found to develop at the proximal end of the cuttings used in this study. At the distal end a similar type of callus formation takes place, but only roots were produced. Attempts to change the polarity of the root by inverting pieces and allowing them to stand in a vertical position in the moist chamber were unsuccessful. Lateral roots appeared occasionally from the sides of the old roots. Goebel (1905) demonstrated shoot formation from the distal end of root cuttings of dandelion when the growth at the shoot pole was arrested by covering with wax or plaster. Wiesner (1892, p. 112) was also able to induce shoot formation

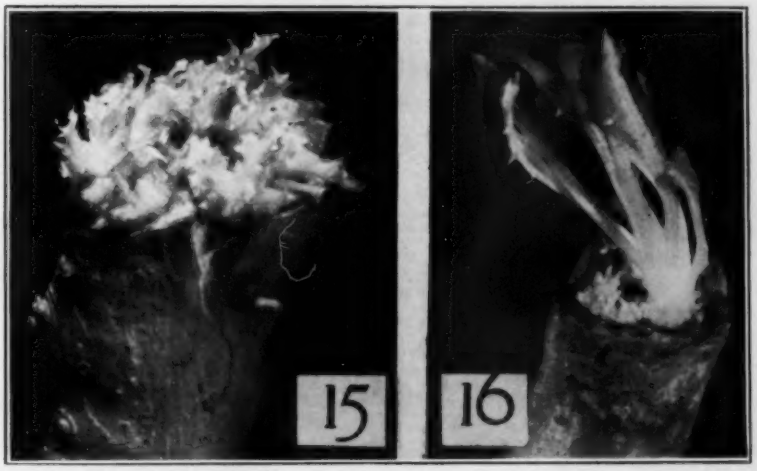


FIG. 15. Side view of root and young shoots after seven days. × 4. FIG. 16. End of cutting showing young shoots after ten days. × 2.5. (Photographs by courtesy of the New Botanical Garden.)

from both ends of root cuttings when they were kept in the dark. I have been unable to duplicate his results. Roots were grown both in the light and the dark, and the callus cut off repeatedly, but cuttings placed in the dark behaved like those placed in the light except for the failure to develop green color.

When fleshy roots were cut lengthwise along the median line both halves developed leafy shoots within 7 days. The new parts originated from phloem parenchyma and appeared in continuous strips on either side of the xylem cylinder.

Leaves of dandelion plants collected in April and May failed to develop any new parts when placed in moist sand in the greenhouse. They turned yellow and died within a period of two weeks. Stingl (1909) also failed to get regeneration from detached leaves.

SUMMARY

On root cuttings of dandelion in moist chambers proliferating tissue appears in two or three days.

The mature main root consists largely of phloem parenchyma, in which are embedded concentric rings of sieve tubes associated with laticiferous ducts.

The new parts originate from the parenchyma of the secondary phloem.

Shoots are formed at only the proximal end of the cuttings, and roots only at the distal end or occasionally along the sides.

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# METABOLISM OF ASCORBIC ACID IN COWPEA PLANTS

MARY ELIZABETH REID

(WITH EIGHTEEN FIGURES[1])

A study of daily fluctuations in the ascorbic acid content of cowpea plants has been under way in this laboratory during the past four years. Announcement of the project was made previously (Reid 1939). Moldtmann (1939) reported a diurnal variation in the percentage content of ascorbic acid in leaves of *Fagus silvatica* and *Lamium album*. Increases occurred during the day and losses at night. Smith and Gillies (1940) found a maximum value in potato leaves in the early forenoon and minimum values toward the end of the night. Considerable fluctuations also occurred during the day. Kohman and Porter (1940) observed similar diurnal alternations in the tops of tomato plants. The results in these latter studies were presented also on a percentage basis. Reid (1940) reported briefly on fluctuations in the total quantity of ascorbic acid in leaves, stems and roots of young cowpea plants. The diurnal changes in ascorbic acid values were also determined on a percentage basis.

The first experiment herein described was conducted to determine the effect of the normal daily light and dark periods of July and early August upon the accumulation of ascorbic acid and growth of young cowpea plants. The effect of twenty-four-hour dark-periods immediately preceding the ascorbic acid assays was studied in the second experiment. A study was also made of the ability of cotyledons and of different-sized particles obtained from broken cotyledons to synthesize ascorbic acid when subjected to conditions favorable to germination.

## PROCEDURE

Cowpea seedlings were grown in washed white sand, some with and some without a mineral nutrient solution. Ascorbic acid determinations by the indophenol method[2] were made daily on each of the two types of cultures. In the first experiment plants were washed free of sand at 4:15 a.m., the end of the dark period and others at 7:00 p.m., the end of the light period. The leaves, roots, stems, and cotyledons were weighed and analyzed sepa-

[1] Figures 12–18 are published at the expense of the author.

[2] The validity of the results obtained in these tests depends upon the supposition that the reducing action of the plant tissue extracts is to be attributed entirely to ascorbic acid. The titrations were made rapidly and the readings were taken when the end-point color was maintained for approximately 20 seconds. Considering the speed of the reaction and the relatively low pH of the extracts, there is no obvious reason for assuming that other substances were involved in the reaction.

rately. In most of the tests ten plants were assayed. In the second experiment plants were prepared for the tests each day at 1:00 p.m., at which time one culture with and one without added nutrients were analyzed. Similar cultures were placed in a dark chamber and assayed for ascorbic acid twenty-four hours later. This procedure was repeated each day throughout the experimental period.

## OBSERVATIONS

The ascorbic acid content and the green weight of young plants which received mineral nutrients are shown in figure 1 and those of plants grown without added nutrients in figure 2. In both series the ascorbic acid increased in the plants during the dark periods as long as the stored food reserves in the cotyledons lasted, which was until the fifth day. After this time no gains were found during the dark period. On the contrary, there were losses after the fifth day in plants which received mineral nutrients and after the sixth day in those grown without added nutrients. Losses of as much as 17 per cent of the total ascorbic acid were found in the plants receiving mineral nutrients and of 8 per cent in those without added nutrients. Since there is greater steepness of the curve representing the ascorbic acid content of the fertilized plants and also since more disappeared at night, these plants must have synthesized considerably more of the vitamin than the unfertilized plants. The green weights of the former were also greater. In both groups the green weights tended to increase at about the same rate in the dark periods as in the light. The above results with entire plants were attained by separate analyses of leaves, stems, and roots. The data for the individual organs were as follows:

The leaves of plants receiving mineral nutrients gained in ascorbic acid during the third and fourth nights but from the fifth night until the termination of the experiment, losses, increasing with each succeeding night, were found. The rate of growth of the leaves in the light and dark periods tended to be equal (fig. 3). The results with plants grown without added nutrients were similar, although values for both growth and ascorbic acid were lower. Losses in ascorbic acid were also found during the fifth night

**Explanation of figures 1–6**

The green weight and ascorbic acid values at successive stages of development shown in figures 1–9 were determined at the end of each daily light and dark period. The broken portions of the lines represent the light periods and the solid segments the dark periods. Except where the contrary is specially designated, the graphs shown represent the average values in a plant grown with added nutrients.

FIG. 1. Green weight and total ascorbic acid values of entire plant. FIG. 2. Green weight and total ascorbic acid values of entire plant grown without added nutrients. FIG. 3. Green weight and ascorbic acid values of primary leaves. FIG. 4. Green weight and ascorbic acid values of roots. FIG. 5. Green weight and ascorbic acid values of stem. FIG. 6. Total ascorbic acid values expressed on a uniform scale for all organs of a plant.

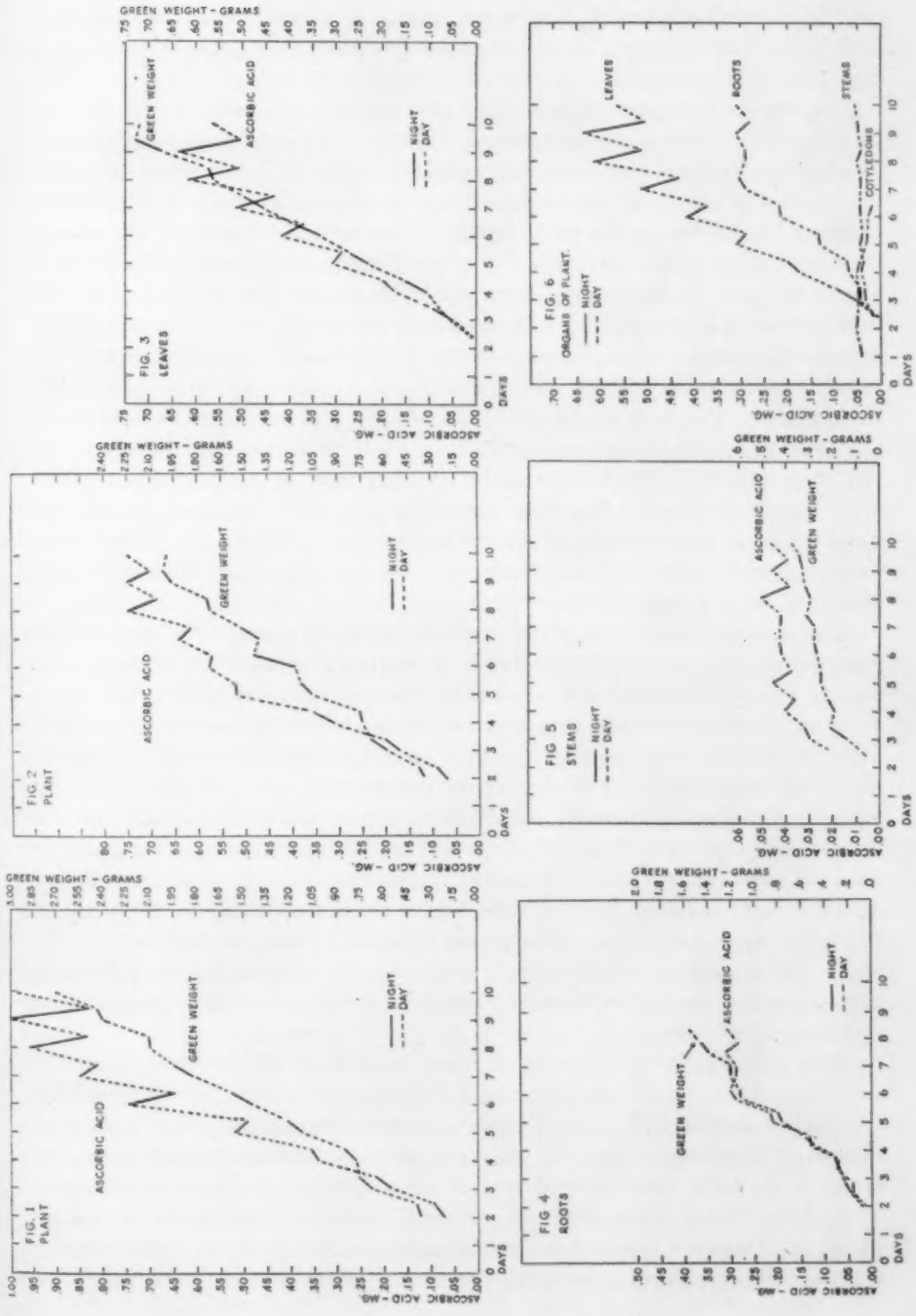
FIG. 1
PLANT
ASCORBIC ACID
GREEN WEIGHT
NIGHT
DAY
GREEN WEIGHT - GRAMS
ASCORBIC ACID - MG.
DAYS
FIG. 2
PLANT
FIG. 3
LEAVES
FIG. 4
ROOTS
FIG. 5
STEMS
FIG. 6
ORGANS OF PLANT
LEAVES
ROOTS
STEMS
COTYLEDONS

and on each night thereafter. The lag in rate of ascorbic acid accumulation and in increase in green weight between the ninth and tenth days occurred when the seedling leaves were fully developed.

Figure 4 shows the green weight and ascorbic acid values of the roots of the plants which received mineral nutrients. A fairly close relation is to be observed between the green weight and ascorbic acid content of the roots at successive stages of development up to the ninth day. A divergence between the values as the roots became older was also found in the plants grown without added nutrients. The green weights and ascorbic acid tended not to increase appreciably during the dark periods. In the plants grown without added nutrients and which consequently (Kraus and Kraybill 1918) had accumulated a much larger supply of mobilizable carbohydrates, there was a tendency for increases in both weight and ascorbic acid during the dark periods. Previous studies have shown a similar relation between available carbohydrate supply and growth of roots of tomato cuttings kept in darkness (Reid 1926). Decreases in ascorbic acid in roots did not occur with regularity during the dark period in any of the tests. It is possible, however, that translocation of the vitamin to the roots occurs in darkness or that some synthesis occurs within the root with the result that losses and gains are approximately balanced.

The ascorbic acid and green weight values of stems of plants which received mineral nutrients are shown in figure 5. No gains in ascorbic acid during the dark period were noted after the food reserves in the cotyledons were exhausted. Increases in green weight tended to be continuous and of about the same magnitude in the light and dark periods after the fourth day. The irregularity in the curve on the seventh day may be caused by slight variations in intensity of illumination of different cultures at the time of active growth of the hypocotyls. The light- and dark-period ascorbic acid values of all organs of the plant receiving added nutrients are shown on a uniform scale in figure 6. The leaves contained about 65 per cent of the total quantity of the vitamin and the roots about 30 per cent in the eight- to ten-day stage of development. As the plants became older the proportion in the leaves became relatively greater and that in the roots correspondingly less.

The great difference resulting from expression of the metabolism of ascorbic acid as total amount or as milligrams per gram may be noted by comparing figures 1, 7, and 8. Figure 7 shows the ascorbic acid values of the entire plant and of the top only expressed as milligrams per gram and figure 8 the total ascorbic acid and green weight of the top excluding the cotyledons. The much smaller size of the diurnal fluctuations in the entire plant, as compared to those in the tops only, result from the fact that the roots, which constitute a relatively large proportion of the green weight of

the entire plant, did not show fluctuations with regularity. Figure 9 shows the ascorbic acid content of the primary leaves on a percentage basis. The slope of the curve is strikingly different from that representing the progressive changes in total content (fig. 3). There was a marked gain in concentration and in total quantity of the vitamin on the third day when the chloroplast-containing mesophyll tissue was developing rapidly. Rapid growth on the fourth night resulted in a marked decrease in ascorbic acid on a percentage basis. The total values show that actually a considerable gain occurred during this time. During the light period on the fourth day only a very slight gain on a percentage basis was found whereas the total quantity in the leaves increased markedly. On the fifth night a very large loss of the vitamin on a percentage basis, undoubtedly caused chiefly by a rapid increase in the water content, and a very slight loss on a total basis occurred. During the light period on the fifth day and on each succeeding day there was a considerable increase on a percentage basis. Greater losses at night than gains during the day in vitamin concentration cause the downward trend of the percentage curve and smaller losses at night than gains during the day are responsible for the upward trend of the curve representing the absolute amounts of ascorbic acid. In plants having many full-grown leaves the influence of growth of a few young leaves during the night on the vitamin C percentage value of the entire mass of leaves would be comparatively slight. There would, however, tend to be an appreciable error as a consequence of the gain in water content which is known to take place at night even in fully grown leaves under the general atmospheric conditions of summer. For example, Lloyd (1913) found from 7 to 15 per cent higher water content at sunrise than in the afternoon in the leaves of cotton plants at Auburn, Alabama, and Tucson, Arizona; Miller (1917) reported a 5.4 per cent higher average water content in corn and 5.9 per cent higher value in kafir between 1:00 and 5:00 a.m. than between 1:00 and 3:00 p.m.

In view of these considerations it seems obvious that only by measurements of the absolute amounts of ascorbic acid either in the entire plant or in individual organs can the magnitude of diurnal changes in its content be shown with a fair degree of accuracy. When plants are considered in terms of their nutritive value with respect to vitamin C, expression of their ascorbic acid value on a percentage basis is both valid and practical but in terms of the performance of the plant itself it is not of any considerable value except when accompanied by an evaluation of the vitamin in terms of absolute amounts.

Figures 10 and 11 show the results obtained in the second experiment in which plants kept in darkness for twenty-four hours previous to testing are contrasted to those subjected to the normal day and night periods.

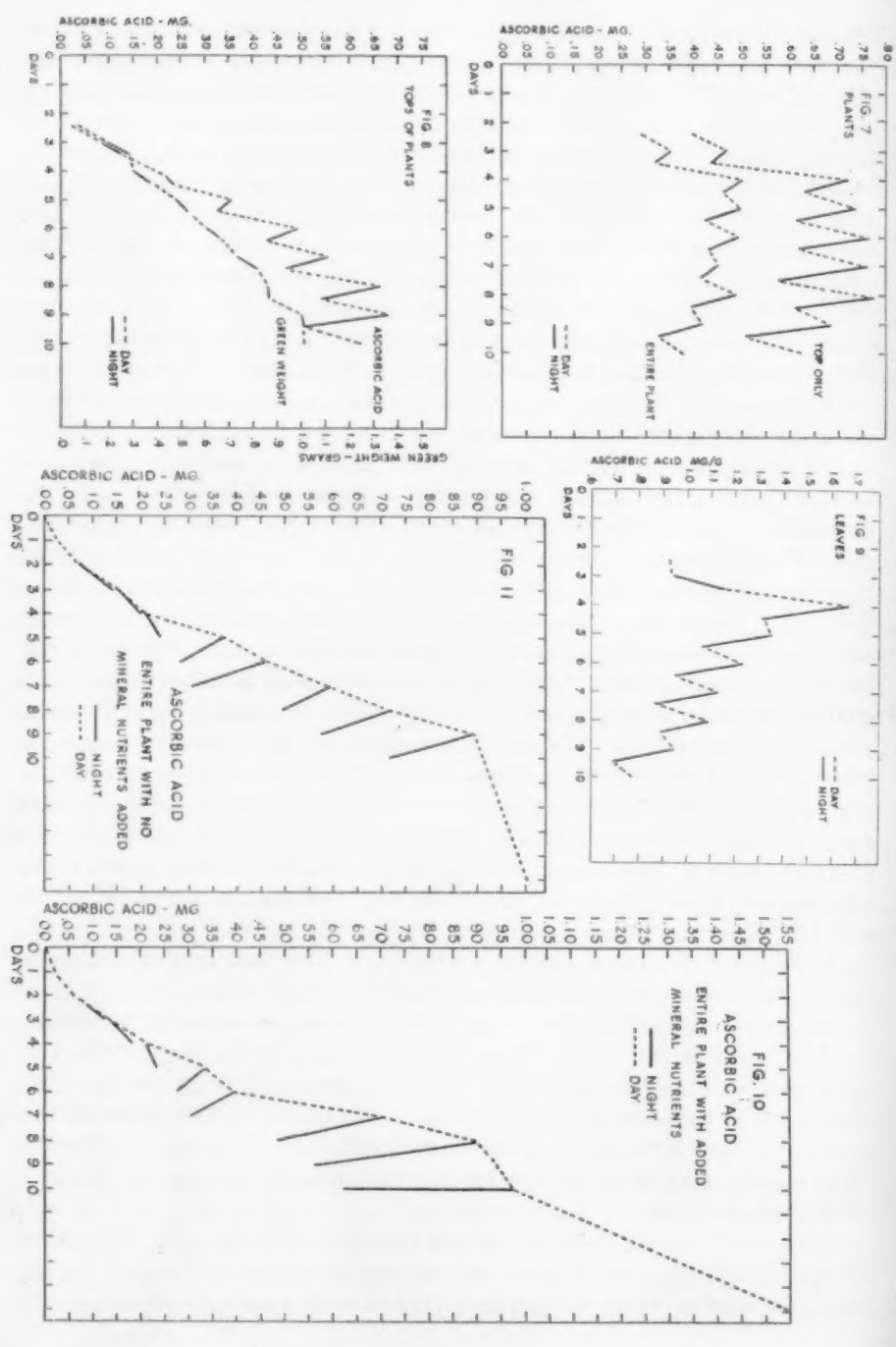
FIG. 7
PLANTS
ASCORBIC ACID - MG.
DAYS
TOP ONLY
ENTIRE PLANT
DAY
NIGHT
FIG. 8
TOPS OF PLANTS
GREEN WEIGHT
ASCORBIC ACID
GREEN WEIGHT - GRAMS
FIG. 9
LEAVES
ASCORBIC ACID MG/G
FIG. 11
ENTIRE PLANT WITH NO MINERAL NUTRIENTS ADDED
FIG. 10
ASCORBIC ACID
ENTIRE PLANT WITH ADDED MINERAL NUTRIENTS

The broken line shows the ascorbic acid content of plants subjected to the normal daily periods of daylight and darkness. The points at the free ends of the solid lines show the ascorbic acid content of the plants kept in darkness for twenty-four hours. The points are connected by the solid lines with the points representing the approximate content of the plants at the time they were placed in the dark chamber. Hence a slope upward of these lines represents a gain in ascorbic acid during the dark period and a slope downward represents a loss. It may be noted (fig. 10) that as in the previous experiment an increase in the total quantity per plant occurred each day until the stored reserves in the cotyledons were exhausted. After this time there were losses during the dark period. The plants grown without added nutrients yielded similar results, although growth was less rapid and losses of ascorbic acid during the dark period were smaller (fig. 11). A lowering of the vitamin C content by as much as 28 per cent in the plants which received nutrients and of 20 per cent in those not so supplied was found. These latter plants contained considerable starch at the end of the dark period. Hence, if plants form ascorbic acid from stored carbohydrates, it appeared that those employed in these tests lacked the capacity, at least to a measurable extent, to utilize their stored carbohydrates in the synthesis of the vitamin during periods of darkness.

The green weights of leaves, stems, and roots all increased during the dark period (figs. 12, 14, 16) and the gains were coincident with losses in the total quantity of ascorbic acid in the plant. Analyses of the vitamin C content of the individual organs revealed that the greater portion of the total loss during the twenty-four-hour dark period occurred in the leaves (fig. 13). However, in proportion to the total quantity present, the losses in the stems were of equal magnitude (fig. 15). The roots tended to show neither definite gains nor losses of ascorbic acid after the plants became independent (fig. 17).

Attempts were made to determine if all or part of the loss of ascorbic acid during periods of darkness may result from conversion of the vitamin to

**Explanation of figures 7–11**

FIG. 7. Milligrams per gram fresh weight of ascorbic acid in entire plant and of top only. FIG. 8. Total ascorbic acid and green weight of top. FIG. 9. Milligrams per gram fresh weight of ascorbic acid in leaves.

In figures 10–17 the broken lines connect points representing ascorbic acid or green weight values of plants grown under normal daily alternations of light and darkness; the points at the free ends of the solid lines represent the values obtained with plants kept in darkness twenty-four hours previous to testing. Each point is connected by the solid lines with the point representing the approximate value of the culture at the time it was placed in the dark chamber. Except where the contrary is specially indicated, the graphs represent the average values in a plant grown with added nutrients.

FIG. 10. Ascorbic acid values in entire plant. FIG. 11. Ascorbic acid values in entire plant grown without added nutrients.

the dehydro form (Bessey 1938). Since the results were not conclusive the problem will be given further study. Moldtmann (1939) found no accumulation of dehydroascorbic acid following periods of darkness in the plants which he studied.

Tests have also been made to determine the course of ascorbic acid accumulation in cowpea seeds during the first twenty-four-hour period of germination. Seeds were supplied with moisture and kept at a temperature of 29° C. Ascorbic acid assays were made at two-hour intervals. Figure 18 shows the results. An indophenol-reducing action of the extracts was observed after a period of four hours and a considerable increase in the magnitude of the reaction was observed by the tenth hour. It is of interest to note that this followed the attainment of maximum absorption of water by imbibition. The increase in green weight after the fourteenth hour is doubtlessly a consequence of growth. At the end of the twenty-four-hour period the sprouts were about 1 centimeter long.

It was observed in other experiments that detached cotyledons placed on moist filter paper in Petri dishes and incubated for 20 hours at 29° C. produced almost as much ascorbic acid as intact seeds incubated similarly. The cotyledons of old seeds which had lost viability produced no ascorbic acid when placed under conditions favorable to germination. When a fine powder produced by grinding a weighed quantity of the cotyledons was incubated there was no rapid indophenol-reducing activity such as is characteristic of ascorbic acid. There was, however, a very slight but slow reaction with a fading end-point. These latter results were not in agreement with observations reported by Pereverzev (1937) who found that crushed cotyledons of legumes retained the ability to form vitamin C. Further tests with cowpea cotyledons were conducted to determine the reason for the discrepancy between the present and Pereverzev's results. Cotyledons cut with a scalpel so as to form a mixture of fine and coarse particles, when incubated under conditions favorable to germination, were found to have an ascorbic acid content almost equal to that of entire cotyledons. These results suggested that the size of particles into which the cotyledons were broken might be an important factor in causing the differences in the above mentioned tests.

A series of tests was conducted with cotyledons crushed and sorted into different sized particles. In each of the tests three grams of the material were placed on a double layer of filter paper in a Petri dish, and 5 cubic centimeters of distilled water added. The cover of the dish was raised slightly by

**Explanation of figures 12–18**

FIG. 12. Green weight of leaves. FIG. 13. Ascorbic acid content of leaves. FIG. 14. Green weight of stems. FIG. 15. Ascorbic acid content of stems. FIG. 16. Green weight of roots. FIG. 17. Ascorbic acid content of roots. FIG. 18. Green weight and ascorbic acid content of seeds placed in germinators kept at 29° C. and tested at two-hour intervals during the first twenty-four hours.

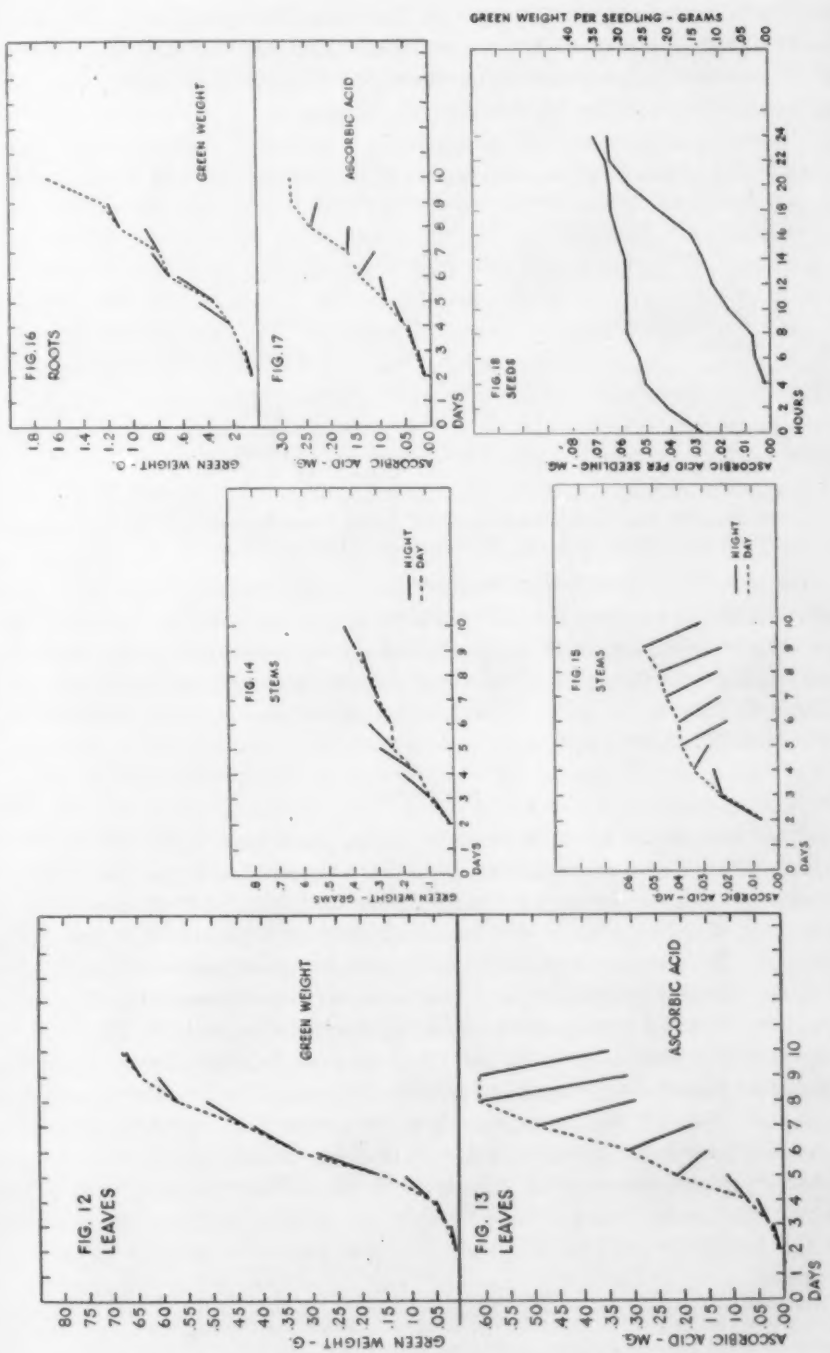
FIG. 12
LEAVES
GREEN WEIGHT
GREEN WEIGHT - G.
FIG. 13
LEAVES
ASCORBIC ACID
ASCORBIC ACID - MG.
DAYS
FIG. 14
STEMS
NIGHT
DAY
GREEN WEIGHT - GRAMS
FIG. 15
STEMS
NIGHT
DAY
ASCORBIC ACID - MG.
DAYS
FIG. 16
ROOTS
GREEN WEIGHT
GREEN WEIGHT - G.
FIG. 17
ASCORBIC ACID
ASCORBIC ACID - MG.
DAYS
FIG. 18
SEEDS
GREEN WEIGHT PER SEEDLING - GRAMS
ASCORBIC ACID PER SEEDLING - MG.
HOURS

a wedge of folded paper to provide for aeration. After incubating the culture at 29° C. for 15 hours, ascorbic acid assays were made. From the data presented in table 1 it appears that size of the cotyledon particles is a factor influencing the capacity for ascorbic acid synthesis.

TABLE 1

*Effect of comminution of cotyledons into different sized particles upon their ability to synthesize ascorbic acid when exposed to conditions suitable for germination*

| Screen | Size of opening | Total ascorbic acid | Ascorbic acid |
|---|---|---|---|
| | *mm.* | *mg.* | *mg./100 g.* |
| Entire cotyledons | ..... | 0.269 | 8.94 |
| ½ normal size | ..... | 0.246 | 8.20 |
| 10[a] | 2.00 | 0.267 | 6.85 |
| 20 | 0.84 | 0.196 | 5.10 |
| 40 | 0.42 | 0.048 | 1.60 |
| 60 | 0.25 | 0.016 | 0.54 |
| 80 | 0.177 | 0.008 | 0.27 |
| Over 80 | ..... | Possibly a trace | ..... |

[a] The data for each screen represent the values obtained with particles retained by that mesh but which passed through the openings of the preceding screens.

The results of these different experiments with cotyledons show (1) that the vitamin can be generated from a precursor stored within the cotyledons and that an influence from the plumule or radicle is unnecessary, and they suggest, furthermore, that vital processes are probably involved since the synthesizing capacity is lost when the tissues are finely pulverized, and also when viability is lost by aging.

## DISCUSSION

After the plants used in these tests had exhausted the reserves in the cotyledons they lacked the capacity during the night to synthesize as much ascorbic acid as was being used, with a resultant loss. In fact, they may not have synthesized any, or the decrease may have represented the excess of loss over gain. The results show conclusively that ascorbic acid is metabolized in darkness. Similar metabolic processes probably occur also in light but they cannot be detected readily because of rapid synthesis in light. The fraction assayed constitutes supposedly the unusued portion only. Hence accumulation is measurable by present techniques but not the full magnitude of synthesis. The fate of the disappearing portion is not known with certainty. It may be destroyed in respiration or it may be used in some special type of synthesis. Experiments with plants kept at different temperatures have shown that it is lost during dark periods only when the temperature is high enough to permit growth, and that the more rapid the growth the greater the loss.[1]

[1] These results will be described in another publication dealing with the effects of temperature on ascorbic acid accumulation in cowpea plants.

The failure of the plants employed in these experiments to increase their ascorbic acid in darkness following exhaustion of the reserves stored in the cotyledons suggests that the capacity to convert carbohydrates to ascorbic acid in darkness is very weak or may be entirely lacking. The ability to accumulate the vitamin in darkness during the period of utilization of the reserves in the cotyledons may be explained (1) by the storage during development of the seed of a closely related precursor which may be mobilized and reconstituted during germination or (2) by a somewhat different type of enzymatic action in the young seedling which may permit the conversion of stored carbohydrates such as starch to ascorbic acid. This capacity may be lacking or at least is relatively weak in the actively growing plant after it becomes independent of the cotyledonary reserves.

There is insufficient evidence to furnish a conclusive answer as to which, if either, of these hypotheses is correct, but there are, however, certain arguments in favor of a synthesis of a closely allied precursor. The relatively prompt reappearance of the vitamin when moisture is supplied also suggests a conversion from a closely related form. Previous study has shown that ascorbic acid accumulates in growing cowpea seeds approximately as long as the green weight continues to increase (Reid 1937). More recent investigations (unpublished data) have shown a similar relation in developing soybeans. Parsons (1920) pointed out that the vitamin might possibly pass into an inactive state on aging or drying and that it might be reconstituted in such physiological processes as the sprouting of seeds. Little attention has been given to this suggestion, however, since up to the present time there has been no obvious reason for questioning the ability of the plant to convert some of the mobilizable carbohydrates into ascorbic acid. Moreover, there has been considerable experimental evidence to support the viewpoint of a fairly direct conversion. Plants kept in darkness have been shown to increase their indophenol-reducing activity when supplied with sugar solutions of several types (Ray 1933, Reid 1938, Moldtmann 1939, Rubin *et al.* 1939, and others). There is no assurance in the reported tests, however, that the cultures were kept in a sterile condition. It is possible that micro-organisms may have produced indophenol-reducing substances other than ascorbic acid. Antiscorbutic tests of the product responsible for the increasing reducing activity in plants under the conditions described have not been reported. It is evident that further study is required before conclusions may be drawn on the ability of plants to convert carbohydrates into ascorbic acid. It is possible that they may be of use in such a synthesis but that the process is not so direct as has been postulated.

## SUMMARY

Ability to increase the total ascorbic acid, as determined by reduction of

indophenol, during periods of darkness continues as long as the stored reserves in the cotyledons remain available.

After this time the plant lacks the capacity to use carbohydrates in the synthesis of the vitamin, or, if the conversion occurs, the rate is so slow that the metabolic loss masks the synthesis.

The loss is accompanied by growth. This suggests that the ascorbic acid may be destroyed in respiration or may be used in some special type of synthesis.

The possibility is discussed that the net loss or gain may not represent the magnitude of ascorbic acid synthesis or loss.

Evidence is presented which shows that the net magnitude of the diurnal fluctuations in ascorbic acid can be shown only by measurements of absolute amounts in individual organs or in the entire plant.

It is suggested that the synthesis of ascorbic acid during the dependent phase of growth is effected by the reconstitution of a closely related precursor stored in the seed during its development.

An influence of the plumule or radicle is unnecessary to effect synthesis of ascorbic acid in the cotyledons. The ability of crushed cotyledons to synthesize the vitamin appears to be dependent upon size of the particles.

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# CALCIUM AND PHOSPHORUS AS THEY INFLUENCE MANGANESE IN FORAGE CROPS[1]

WM. A. ALBRECHT AND N. C. SMITH[2]

(WITH FIVE FIGURES)

Plant physiology has regularly recognized the significance of the degree of acidity of the soil, but has not yet clearly interpreted its significance in plant nutrition. Emphasis on lime additions to the soil for its modification of the degree of soil acidity has overshadowed attention to calcium as a nutrient, and/or as it serves in bringing other nutrients into the plant. Because the degree of hydrogen-ion saturation of the colloidal fraction of the soil is mainly the reciprocal of the loss therefrom of calcium ions, we have been inclined, in cases of crop failure, to attribute causal significance to the wrong one of these reciprocal factors. Studies of the significance of calcium in nitrogen fixation and plant nutrition were undertaken to determine more accurately the role of calcium in crop growth. The separation of its role in changing soil reaction from that in serving as plant nutrient, and in mobilizing other nutrients into the crop, served as a challenge. Its relation in this last respect to manganese in particular, one of the micronutrient elements, was chosen for study.

The importance of manganese in the nutrition of plants (5, 12, 13, 16) and of animals (9, 15, 18) has been established well enough to warrant attention to it in soil fertility. Its disturbed delivery to plants by soils near the neutral or alkaline reactions has been recognized (8, 11, 17). Such irregularities put the common practice of liming a soil for the sake of neutrality into the danger zone of general manganese shortage for plants in our extensive agricultural soils. Since calcium as a nutrient item, particularly for nitrogen fixation by legumes, has been separated from its effects in modifying soil acidity (1, 2, 3), the following study attempted to determine manganese movement into the crop when calcium modified the soil reaction as contrasted to that when calcium served as a nutrient without changing the reaction of the entire soil body in root contact. Phosphates were tested similarly as to their influence on the manganese.

## PLAN OF STUDY

The soil used was the surface layer of a well weathered prairie soil of

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the Kansan glaciation, one of the planosols,[3] known as the Putnam silt loam. It is of acid reaction, pH 5.5, relatively low in organic matter and phosphorus, and developed to the point of having a marked clay concentration in the upper subsoil. Its exchange capacity is approximately 20 M.E. per 100 gms. of soil. This is usually saturated to about 50 per cent with hydrogen. The more common legumes will produce only poor yields on this soil.

*Amounts of treatments applied.* Pot cultures were grown in quintuplicates of two legumes, sweet clover (*Melilotus alba*) and lespedeza (*Lespedeza stipulacea*), and of two non-legumes, bluegrass (*Poa pratensis*) and redtop (*Agrostis alba*). The soil treatments consisted of separate additions of calcium carbonate, of calcium phosphate and of sodium phosphate, and of joint additions of the calcium carbonate and calcium phosphate. The amounts of calcium carbonate applied to the soil were such as (a) would exchange slightly more than one-half of the replaceable hydrogen in one-fourth of the soil; and (b) would more than replace the total exchangeable hydrogen in this smaller soil portion. The phosphates were used in amounts that represented (a) liberal applications in practice, and (b) twice this amount.

*Placement of treatments.* The applications of calcium carbonate were made by distributing it (a) throughout all the soil in the pot, and (b) into the surface one-fourth of the soil. The calcium phosphate was applied similarly. The sodium phosphate was put into only the surface or one-fourth. The joint applications of calcium carbonate and calcium phosphate were used in the four possible combinations as amounts, and were placed into the surface or one-fourth of the soil only.

These placements of the calcium carbonate provided, in the first place, two different, but low, degrees of calcium saturation or of partial neutralization of the entire soil volume available to the plant. In the second place, they provided soils in which the surface areas alone had two different degrees of calcium saturation, namely one with more than 75 per cent and one in excess of complete calcium saturation, while three-fourths of the soil volume remained untreated. For simplicity's sake, these distributions of the additions to the soil may be spoken of by numbers as follows:

Treatment No. 1—Small amount throughout the entire soil volume.
Treatment No. 2—Large amount throughout the entire soil volume.
Treatment No. 3—Small amount through one-fourth or surface of the soil.
Treatment No. 4—Large amount through one-fourth or surface of the soil.

The phosphate additions represented different and higher degrees of saturation by the phosphate in the surface portion of the soil, and two different, but much lower, degrees of saturation when applied throughout the entire soil body. The combinations of calcium carbonate and calcium

[3] According to *Soils and Men*, U.S.D.A. Yearbook of Agriculture, 1938.

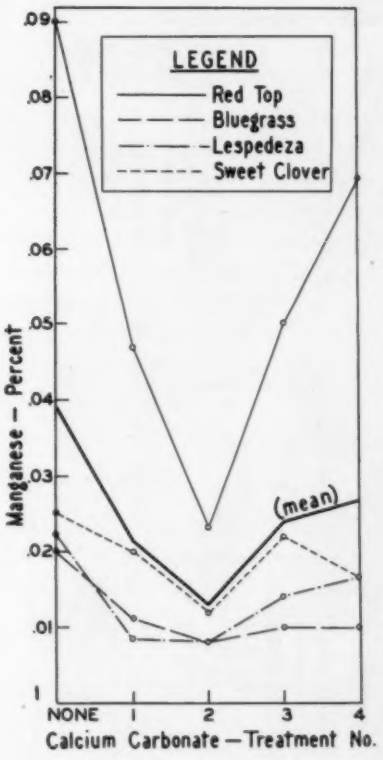
phosphate were used in the surface portion only, and represented the higher degrees of surface soil saturation by each of them. No manganese was applied.

*Analytical methods.* The crops were harvested at intervals of significant top growth so as to simulate grazing. Weight records of the harvests were taken. The crops were submitted to analysis by the standard gravimetric methods for the calcium and phosphorus and to the Lundegardh spectrographic method for manganese, and account was taken of the concentrations and totals in the crops.

## RESULTS

*Results from calcium carbonate.* The most noticeable result was the influence of the increasing amounts of calcium carbonate throughout the entire mass of soil (Treatments Nos. 1 and 2), as they reduced the percentage and the total amount of manganese in the crop; and the influence of the increasing degree of saturation of the surface soil layer by calcium (Treatments Nos. 3 and 4), as it had the very reverse effect. The percentages of the manganese in the crop are shown in figure 1, and the totals of it in figure 2. There is such a pronounced similarity between these two figures as to testify that the manganese responds as a decided nutritional disturbance in the plant metabolism rather than as a variation in manganese amounts simply according to variable plant mass. When the manganese behaviors, as given in figures 1 and 2, are related to the total calcium taken by the crop, as given in figure 3, then this fact becomes more evident. In Treatments Nos. 1 and 2 by limestone, which were lessening the soil acidity to greater degrees and were giving large calcium intake by the crop, there were decided decreases in the manganese concentrations of and intake by the crop. Yet in Treatments Nos. 3 and 4, which saturated the upper portion of the soil more completely so as to change the soil reaction much more in that limited soil volume and to deliver still greater amounts of calcium into the crop, there was the reverse relation between the manganese and the calcium, and gave increasing concentrations and total amounts of manganese taken by the crop.

This suggests clearly that an increase in the amounts of calcium carbonate throughout the soil, so as to shift its reaction toward neutrality, served to decrease the movement of manganese into the crop. Increasing the delivery of calcium to the plant by means of the higher saturation in the limited surface soil zone brought increasing delivery of manganese presumably from the remaining three-fourths of the soil given no calcium carbonate. As an agent that removed soil acidity, the calcium carbonate lessened the amount of manganese going from the soil to the crop. As a fertilizer that supplied calcium to the crop, it enabled the plants to take greater amounts of manganese from the soil below the zone of calcium fertilization.

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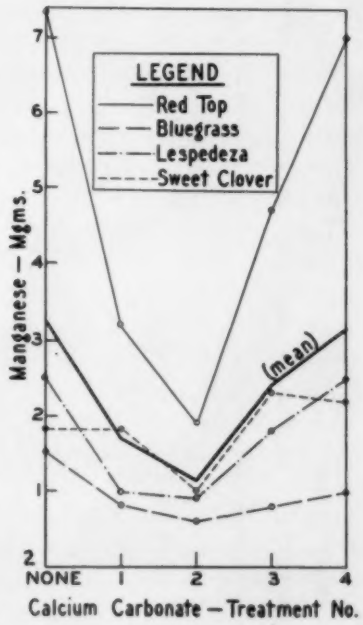
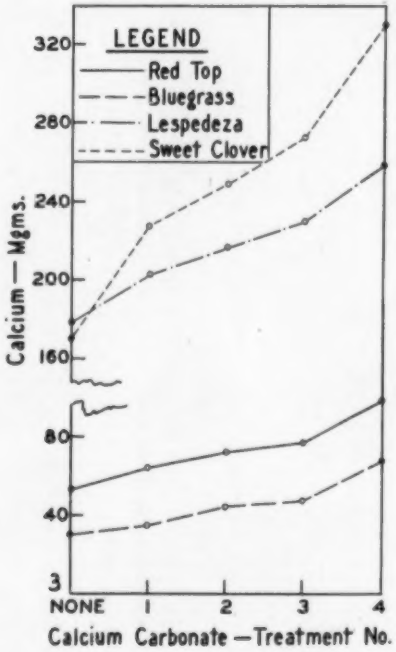



2

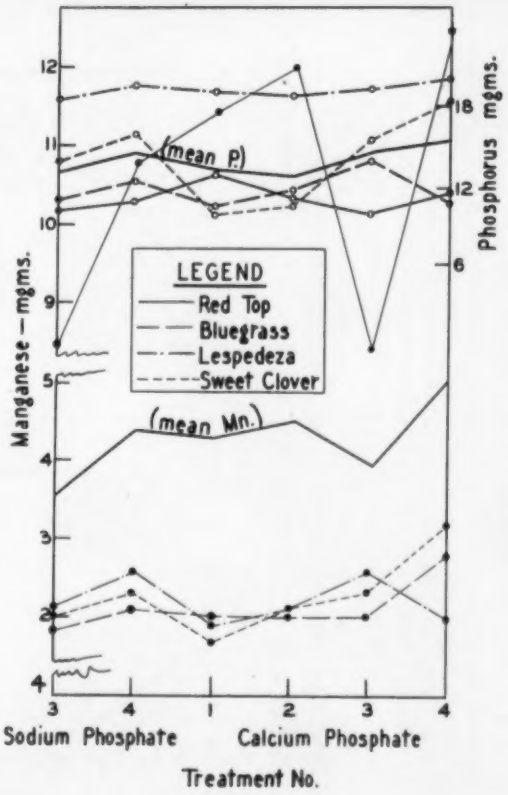
FIG. 1. Percentages of manganese in the different crops as influenced by two different amounts of calcium carbonate applied throughout the soil (Treatments Nos. 1 and 2), and into the surface soil only (Treatments Nos. 3 and 4). FIG. 2. Total manganese in the different crops as influenced by two different amounts of calcium carbonate applied throughout the soil (Treatments Nos. 1 and 2), and into the surface soil only (Treatments Nos. 3 and 4).

Possibly an increased or modified microbiological action as a competitor with the plant for the manganese (7, 10), may be the explanation for the reduction in manganese within the crop when calcium carbonate was distributed throughout the soil. This is suggested by the fact that the smaller application (Treatment No. 1), was relatively more disturbing than the larger one (Treatment No. 2), as is shown by the graph as the mean for all four crops either as percentage or as total manganese in figures 1 and 2. These studies did not aim to separate the microbial from the simple chemical effects.

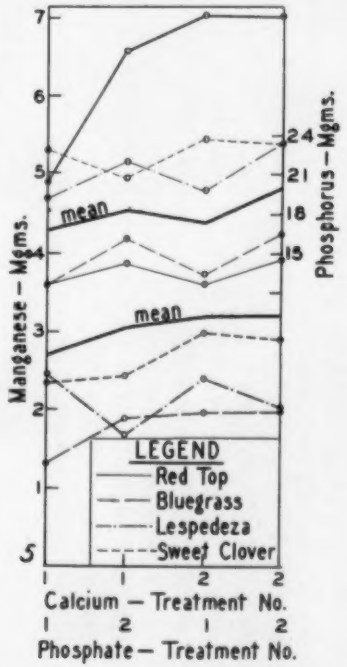
Such facts bring to attention the need to consider whether our use of calcium carbonate, or limestone, for the purpose of correcting soil acidity


LEGEND
Red Top
Bluegrass
Lespedeza
Sweet Clover
Calcium — Mgms.
320
280
240
200
160
80
40
3
NONE 1 2 3 4
Calcium Carbonate — Treatment No.

3


(mean P.)
LEGEND
Red Top
Bluegrass
Lespedeza
Sweet Clover
(mean Mn.)
Manganese — mgms.
Phosphorus mgms.
12
11
10
9
5
4
3
2
18
12
6
3 4 1 2 3 4
Sodium Phosphate
Calcium Phosphate
Treatment No.

4


mean
mean
LEGEND
Red Top
Bluegrass
Lespedeza
Sweet Clover
Manganese — Mgms.
Phosphorus — Mgms.
7
6
5
4
3
2
1
24
21
18
15
1 1 2 2
Calcium — Treatment No.
1 2 1 2
Phosphate — Treatment No.

5

is the most effective service by this soil treatment that is now undergoing wider adoption as a regular farm practice. They suggest that calcium must be put into the fertilizer category, and that excessive applications prompted by low cost or unbridled enthusiasm may bring disappointments.

*Results from phosphates.* The influence of the single phosphate soil treatments on the manganese intake into the crop was wholly positive, in that as more phosphate was given, either throughout the entire soil mass or into the surface soil only, there was increasing manganese in the harvest. These data are brought together in figure 4, which gives the totals for manganese and for phosphorus taken by the plants per pot.

Increasing the sodium phosphate in the upper soil portion increased the manganese taken. Putting the calcium phosphate into the top soil (Treatments Nos. 3 and 4), was more effective in bringing both manganese and phosphorus into the harvest. This is shown by the highest points on the graphs at the right side where the double dosage of calcium phosphate in the surface soil is indicated. The positive relation between the total manganese and the total phosphorus in the crop is shown more clearly by the close parallelism between the graphs representing the respective means for all crops. It points to an increase of manganese that was parallel with increased crop yield.

*Results from calcium carbonate combined with calcium phosphate.* When the joint applications of calcium carbonate and calcium phosphate are observed for their influence on the manganese harvested in the crop, this is slightly greater per unit of phosphorus taken by the crop than was the case where phosphates were applied singly. This is shown in figure 5, more particularly in the graph for the mean of all crops combined. The offering of more of each of these soil treatments so as to grow heavier crops increased the manganese taken from the soil. This suggests that the use of the manganese was correlated with the better or increasing growth through calcium and phosphate treatments.

Seemingly, then, the manganese was not a limiting factor in this Putnam silt loam. Its consumption by the plant from this soil was hindered, in some measure, by the phosphorus shortage, but more by the calcium shortage. It was when both calcium and phosphorus were applied that the largest

**Explanation of figures 3–5**

FIG. 3. Total calcium in the different crops as influenced by two different amounts of calcium carbonate applied throughout the soil (Treatments Nos. 1 and 2), and into the surface soil only (Treatments Nos. 3 and 4). FIG. 4. Total manganese and phosphorus in the different crops as influenced by different amounts of phosphates applied throughout the soil (Treatments Nos. 1 and 2), and into the surface soil only (Treatments Nos. 3 and 4). FIG. 5. Total manganese and phosphorus in the different crops as influenced by two different amounts of calcium carbonate and calcium phosphate used in their possible combinations in the surface soil. (Lighter lines = P. Heavier lines = Ca.)

crops were grown, and that the larger amounts of manganese were taken from the soil.

*Manganese contents of the different crops.* The contents in manganese of the different crops are worthy of note. They are in agreement with analyses by others, particularly the high value for redtop (6, 14), and suggest that manganese is another help in fitting crops into the general ecological array of plants according to composition (4). The variations in concentrations and totals in the different crops are assembled in table 1.

TABLE 1

*Concentrations and totals of manganese in the different crops.*
*(Ranges for different soil treatments)*

| Crops | Concentrations, per cent | | | Totals, mgms. | | |
|---|---|---|---|---|---|---|
| | Soil treatments | | | | | |
| | Calcium carbonate | Phosphates | Calcium carbonate plus phosphate | Calcium carbonate | Phosphates | Calcium carbonate plus phosphate |
| Redtop | .023–.064 | 0.10 –0.14 | .053–.071 | 1.9–7.0 | 0.8–1.3 | 4.9–7.0 |
| Bluegrass | .008–.01 | 0.020–0.024 | .013–.018 | 0.6–1.0 | 1.8–2.8 | 1.3–1.9 |
| Lespedeza | .008–.017 | 0.014–0.018 | .011–.016 | 0.9–2.5 | 1.9–2.6 | 1.7–2.4 |
| Sweet clover | .012–.022 | 0.020–0.026 | .014–.019 | 1.0–2.3 | 1.7–3.2 | 2.3–3.0 |
| Range of variation in concentration | 0.56 | .016 | .042 | | | |

In terms of concentrations of manganese within the crop, redtop is much the highest, reaching 0.14 per cent on soil given calcium phosphate only, and .071 per cent where this treatment was combined with calcium carbonate. It is followed in order by sweet clover with .026 per cent as maximum. Then comes bluegrass with .024 per cent as its highest and lespedeza with .018 per cent as its uppermost concentration of manganese in these trials. When the calcium carbonate was put into the surface soil to give more calcium to the crop, then the concentration of manganese was higher in the lespedeza than in the bluegrass.

When attention is directed toward the total manganeses in the crop, then the redtop is the highest, provided it was given calcium phosphate either singly or combined with calcium carbonate. Here in the case of a crop commonly considered suitable for lime-deficient soils and commonly not given soil treatment, calcium and phosphorus play an influential role in helping it to get manganese from the soil. Sweet clover follows in order as it did for manganese concentrations, particularly for the soil treatments of calcium carbonate and calcium phosphate used jointly. Bluegrass took the largest amounts for this crop when it was given phosphate, while lespedeza behaved similarly.

SUMMARY

The concentrations of manganese within the crop and the totals taken by it from the soil point to a dual role by calcium carbonate in relation to this micro-nutrient in plant nutrition. When calcium carbonate, or limestone, was mixed throughout the soil so as to modify its reaction, then the concentration and total of manganese in the crops were decreased as the application to the soil and the consumption of calcium by the crop increased. When, however, the application was put into the surface soil to increase crop consumption of the calcium even more, then the reverse effect on manganese was manifested, so that the concentration and the total of manganese were increased.

When phosphate was applied to provide increasing concentrations, whether in the entire or in the limited soil volume, then the additional phosphorus for the crop gave increasing manganese harvest, roughly parallel with increased crop growth.

Combinations of lime and phosphate in the limited soil areas aided the crop in taking more manganese.

These studies emphasize the need to consider the beneficial nutritional role of calcium within the plant, on soils such as the type used, in making manganese available for the plant as reflected in the increased concentrations. Then there is need to consider the possible detrimental role of calcium carbonate as it modifies the reaction of the soil or other soil conditions and reduces the manganese taken by the crop possibly to the danger point of manganese deficiency.

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# CYLINDROCHYTRIDIUM JOHNSTONII GEN. NOV. ET SP. NOV., AND NOWAKOWSKIELLA PROFUSUM SP. NOV.

JOHN S. KARLING

(WITH SIXTEEN FIGURES)

During the Christmas holidays, 1940, while duck hunting on the Mount Prospect estate of J. Ambler Johnston which adjoins the Pamunky River in New Kent County, Virginia, I collected samples of water containing vegetable debris from a number of swamps and streams to determine the presence and relative abundance of chytridiaceous fungi during the winter months. These samples were brought to New York and placed in deep glass dishes on a table before an east window in the laboratories at Columbia University and baited with cooked, bleached leaf fragments, root tips, etc., of various grasses in the manner previously described by the author (1934, 1935, 1937). Within a fortnight a large number of fungi and chytrids appeared. The infected grass tissues were then washed thoroughly with distilled water under pressure and transferred to sterile Petri dishes containing charcoal water and bits of cellophane. The latter has proven to be an excellent medium for the growth of these chytrids, as Haskins (1939) has so well shown for other species.

Among the numerous fungi which were trapped in this manner appeared a few thalli of a monocentric chytrid with a cylindrical, stalked or septate sporangium subtended by an extensive catenulate rhizoidal system. Because of the characteristic shape of its sporangium, this chytrid was first believed to be a species of *Clavochytridium* Cox (1939) or *Blastocladiella* Matthews (1937), but subsequent observations showed that it differs from these genera by one outstanding and several minor characters. The sporangia of the two above-mentioned genera open by the deliquescence of one or more exit papillae, while those of our fungus are operculate. In addition, its zoospores include one large refringent globule like most other chytrids; those of *Clavochytridium* and *Blastocladiella* contain several minute refractive bodies and resemble the zoospores of other species of the Blastocladiales.

Because of its operculate, cylindrical, and stalked sporangia our species does not belong in either of these or any other known chytrid genera. A new genus is accordingly established to include this chytrid, and because of the characteristic shape of its sporangia the name *Cylindrochytridium* is proposed. The type species is named *C. Johnstonii* in honor of Mr. J. Ambler Johnston of Richmond, my kind and generous host on the occasion of this pleasant holiday in Virginia.

CYLINDROCHYTRIDIUM Karling, gen. nov.

Thallus monocentric and eucarpic. Zoosporangia usually stalked and cylindrical, occasionally sessile, operculate. Zoospores posteriorly uniflagellate, emerging fully formed and lying quiescent in a globular mass at the orifice of the sporangium for a few minutes before swimming away. Rhizoidal system extensive, branched with numerous catenulate spindle-shaped swellings. Resting spores unknown.

Thallo monocentrico, eucarpico; zoosporangiis plerumque stipitatis et cylindricis, aliquando sessilibus, operculatis. Zoosporis a tergo uniflagellatis, maturis emergentibus et globoso cumulo orificio sporangii quiescentibus atque mox natantibus. Rhizoideo systemate extenso et ramoso, cum numerosis catenulatis fusiformibus incrementis. Sporis perdurantibus incompertis.

**Cylindrochytridium Johnstonii** Karling, sp. nov. Zoosporangia hyaline, smooth, thin-walled, usually tubular, cylindrical or slightly clavate, 12–25 $\mu \times 30$–800 $\mu$, occasionally oval, pyriform and sessile; stalk or basal portion usually of the same shape and diameter as the sporangium, but sometimes inflated, vesicular, irregular and apophysis-like. Operculum oval, ellipsoidal, $4 \times 6$ $\mu$–$8 \times 10$ $\mu$, spherical, 4–17 $\mu$; remaining attached to the sporangium or lying loose nearby. Zoospores spherical, 5.6–7 $\mu$, with a large highly refractive globule; flagellum 22–26 $\mu$ long. Rhizoidal system arising

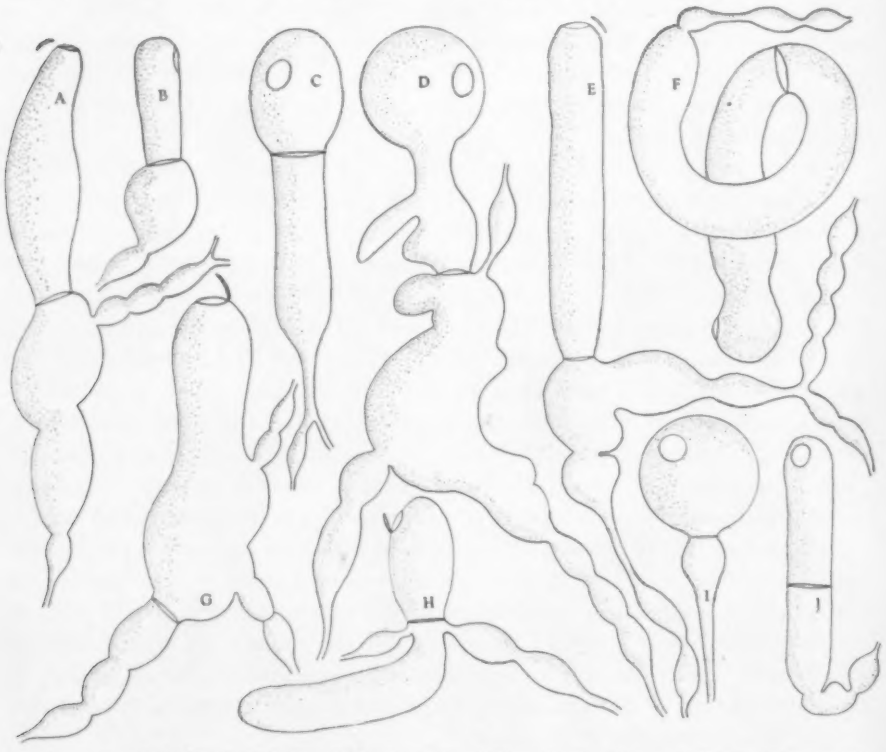



FIG. 1. *Cylindrochytridium Johnstonii*. Variations in the size and shape of sporangia.

from one to several points on the base of the stalk, extending over a radius of 100–1200 μ; spindle shaped swellings 8 × 12 μ–20 × 30 μ, rarely lacking entirely.

Saprophytic in decaying vegetable debris in swamps and streams in New Kent County, Virginia.

Fungus saprophyticus; zoosporangiis hyalinis, levibus, tenui pariete, plerumque tubulosis, cylindricis aut parum clavatis, 12–25 μ × 30–800 μ, interdum ovalibus, pyriformibus atque sessilibus; stipite aut radicali parte plerumque similiformi et -diametro sporangio, sed aliquando inflato, vesiculoso, irregulari et apophysiformi. Operculo ovali, ellipsoideo, 4 × 6 μ–8 × 10 μ, aut sporangio affixo aut liberato adiacente. Zoosporis sphaericis, 5.6–7 μ, magno maxime refractivo globulo praeditis; flagello 22–26 μ longo. Rhizoideo systemate aut uno aut pluribus locis radicali ex parte stipitis oriente, 100–1200 μ; incrementis fusiformibus 8 × 12 μ–20 × 30 μ, quae raro omnino desunt.

The sporangia of this species are predominantly tubular and cylindrical, but they may often vary considerably in shape, as is shown in figures 1A–1J. In some individuals the basal portion or stalk of the thallus may be very irregular (figs. 1A, 1D, 1E, 1G, and 1H), while in others the sporangia are curved and coiled with inflated tips (figs. IF, 13, 14). Oval and obpyriform sporangia with short or elongate stalks may also occur (figs. 1C, 1I, 11, 12). When treated with choro-iodide of zinc, the walls become pale lavender in color, indicating a faint trace of cellulose. In this respect *C. Johnstonii* differs from *Clavochytridium stomophilum* and *B. simplex*.

While the spindle-shaped swellings in the rhizoidal system are usually present, they may be lacking entirely in certain thalli (figs. 1C, 1I, and 12). These swellings were first believed to be due to the expansion of the rhizoids after they had passed through successive cell walls of the grass leaf, but this is apparently not the cause of their formation. In the elongate elements of the vascular strands as many as four swellings were present in a single host cell, which indicates that they are a fairly constant morphological character. The rhizoidal system of *C. Johnstonii* is very similar to that of *Catenochytridium* Berdan (1939) with its compound catenulate apophysis, with the exception that the swellings are usually more elongate and thin-walled. In this latter respect it is more like that of *Clavochytridium*. Whether or not the swellings in the rhizoids of *C. Johnstonii* are to be regarded as constituting a compound apophysis is obviously a matter of personal interpretation. Because of its monocentric, rhizidiaceous, eucarpic thallus, this species apparently belongs in the Rhizidiaceae as this family is now recognized. Since no resting spores have been found, its relation to the other genera of this family is not clear at the present time.

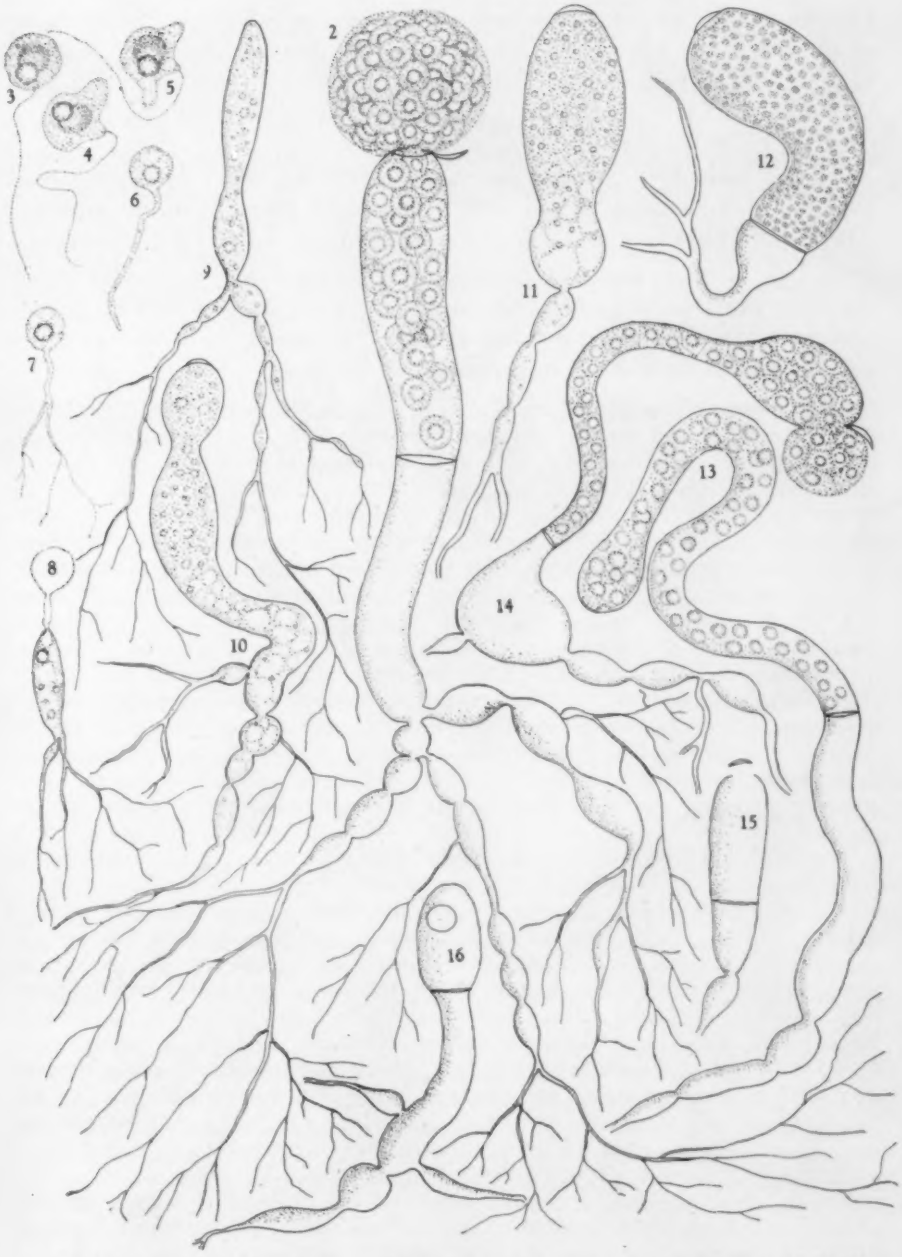
## DEVELOPMENT OF THE THALLUS

The development of *C. Johnstonii* is essentially the same as that of most

monocentric rhizidiaceous chytrids which Sparrow, Couch, Berdan, and others have previously described, and it would be superfluous to describe this process in detail again. The zoospores (fig. 3) are characteristic in shape and activity and possess the well-known large refractive globule and posterior flagellum. The active swimming period lasts from 20 minutes to two hours, during which the zoospores may become intermittently amoeboid (figs. 4, 5). In germination, a fairly thick germ tube is formed (fig. 6) which penetrates the host cell and soon branches once or several times (fig. 7). When the boiled grass tissues are very soft, and at the point of disintegration, the thalli may develop intramatrically, and in such cases the rudiments of the sporangium and stalk begin as an elongate swelling in the germ tube at or near the point of branching (fig. 8). As the swelling increases in size, the zoospore case and a portion of the germ tube eventually disappear. When the thallus occurs partially intra- and extramatrically, the zoospore and a part of the germ tube form the sporangium and stalk after the rhizoidal system has become fairly well established (figs. 9, 10).

As the thallus matures, the protoplasm slowly flows upward into the incipient stalk and sporangium, and as a result the rhizoids and lower portions become increasingly vacuolate (figs. 10, 11) in much the same manner as has been described by Cox for *Clavochytridium*. In the meantime the amount of refractive material has increased and appears in the form of glistening suspended globules, as is characteristic of most rhizidiaceous chytrids. After most of the protoplasm has aggregated in the upper part of the thallus, a cross wall is formed which delimits the sporangium proper from the more or less empty portion and rhizoidal system (fig. 12). After this, the protoplasm becomes coarsely granular in appearance (fig. 12), and in a few hours the large definitive refractive globules appear (fig. 13). Cleavage seems to occur during or shortly after the granular stage, but cleavage furrows are not conspicuous at any stage in living material. The sporangium may remain in the stage shown in figure 13 for several hours, but eventually the operculum is pushed up and the zoospores begin to emerge (fig. 14). The operculum is fairly large and may occur at the apex (figs. 2, 15, 1G, 1H, 1E, 1F) or subapically (figs. 1C, 1D, 1I, 1J, 1r, 16).

If the thallus occurs intramatrically in the soft host tissues, the tip of the sporangium usually projects to the surface so that the zoospores have no difficulty in getting out of the host. In some thalli, however, the zoospores may be liberated directly within the host. The zoospores emerge slowly en masse and are surrounded by a film of fairly consistent fluid or matrix (fig. 2), as in *Endochytrium, Chytridium, Nephrochytrium,* etc. Before all have emerged from the sporangium, the globular mass may break up and the individual zoospores swim away. Those which are left in the sporangium




FIGS. 2–16. *Cylindrochytridium Johnstonii;* showing the development of the thallus and sporangia.

then emerge one by one and swim directly away. In swimming, the zoospores of *C. Johnstonii* behave like those of most other chytrids, and nothing unusual in this respect has been observed. So far, no resting spores have been found, and it is impossible to say at present whether this species is homo- or heterothallic.

In the same host tissues with *C. Johnstonii* occurred another chytrid species which is characterized by an unusually coarse, profuse rhizomycelium and operculate zoosporangia. It is obviously a species of *Nowakowskiella* but differs in several ways from *N. elegans*, *N. ramosum*, and *Septochytrium variable*, and I am accordingly diagnosing it as a new species, *N. profusum*. An intensive cytological study of this species is now in progress, and the results thereof are to be published shortly.

**Nowakowskiella profusum** Karling, sp. nov. Rhizomycelium very profuse, extensive, and richly branched, hyaline, 6-15 μ in diameter; well-defined, septate, spindle-shaped and oval swellings rare or lacking; rhizoids abundant. Zoosporangia terminal or intercalary, hyaline, smooth, rarely apophysate, spherical, 10–45 μ, ovoid, ellipsoid, 8 × 12 μ–20 × 35 μ, clavate, obclavate, 10–22 μ × 15–40 μ, pyriform, obpyriform, elongate, cylindrical, 8 × 25–10 × 50 μ, lobed and irregular. Operculum apical, subapical or lateral, oval and spherical, 4.4 μ–7 μ, remaining attached to the sporangium or lying nearby. Zoospores hyaline, spherical, 4 μ–5.5 μ with a small refringent globule, 0.7–2 μ. Resting spores terminal or intercalary, spherical, 14–25 μ, ovoid, ellipsoid, 10 × 15 μ–14 × 22 μ, truncate, spindle-shaped, and rarely irregular, with a fairly thick, yellowish-brown, smooth wall; content granular with numerous refractive globules; germinating after a short rest period; forming one or two broad, tapering, thin-walled, operculate exit tubes and producing zoospores directly within; or content of spores occasionally growing out and forming a thin-walled zoosporangium on the surface.

Saprophytic in decaying vegetable debris in swamps and streams in New Kent County, Virginia.

Fungus saprophyticus; rhizomycelio maxime profuso, extenso, copiose ramoso, hyalino, 6–15 μ diametro; incrementa definita, septata, fusiformia, ovalia raro habente aut deficiente; rhizoidibus numerosis. Zoosporangiis terminalibus aut intercalaribus, hyalinis, levibus, raro apophysatis, sphaericis, 10–45 μ, ovoideis, ellipsoideis, 8 × 12 μ–20 × 35 μ, clavatis, obclavatis, 10–22 μ × 15–40 μ, pyriformibus, obpyriformibus, elongatis, cylindricis, 8 × 25 μ–10 × 50 μ, lobulatis et irregularibus. Operculo apice aut sub apice aut a latere formato, ovali et sphaerico, 4.4 μ–7 μ, aut sporango affixo aut prope adiacente. Zoosporis hyalinis, sphaericis, 4–5.5 μ, parvo refringente globulo (0.7–2 μ) praeditis. Sporis perdurantibus terminalibus aut intercalaribus, sphaericis, 14–25 μ, ovoideis, ellipsoideis, 10 × 15 μ–14 × 22 μ, truncatis, fusiformibus, et raro irregularibus cum potius crasso, sucineo colorato, levi pariete; contentis granulatis cum permultis refractivis globulis; germinatione post brevem quietem confecta; unum (interdum duo) latum acuminatum, tenui pariete, operculatum tubulum exeuntem gignentibus, zoosporis intra evolventibus, aut contentis sporae interdum emergentibus ut sporangium tenuis parietis in superficie fiat.

That chytridiaceous fungi may occur abundantly during the winter months in Virginia is shown by the large number of additional species which were found in the water and vegetable debris from New Kent County. In addition to the two new species described above, the following known species were found: *Sphaerita* sp. in *Euglena* sp., *Rhizophidium carpophilum* on *Saprolegnia* sp., *R. globosum* on *Cladophora glomerata*, *Endochytrium operculatum*, *Rhizophlyctis Peterseni*, *Catenochytridium carolinianum*, *Nephrochytrium aurantium*, *Cladochytrium replicatum*, *C. tenne* (?), *C. hyalinum* (Berdan, in press), *Septochytrium variable*, and *Nowakowskiella elegans*. All these species are new for Virginia, as far as I am aware.


## SUMMARY

*Cylindrochytridium Johnstonii* is a new monocentric, eucarpic, rhizidiaceous, saprophytic species of the family Rhizidiaceae and occurs in swamps and streams containing vegetable debris in New Kent County, Virginia. It is usually characterized by an elongate tubular, cylindrical or slightly clavate sporangium, an extensive rhizoidal system with numerous catenulate, spindle-shaped swellings, and spherical, posteriorly uninflagellate zoospores with a large refringent globule. Resting spores are unknown.

*Nowakowskiella profusum* is characterized by a coarse, profuse rhizomycelium, terminal or intercalary zoosporangia which are quite variable in size and shape, and yellowish-brown terminal or intercalary resting spore.

In addition to these new species, eleven other chytrids were isolated from water samples from New Kent County, Virginia.



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# CYTOLOGICAL STUDIES IN LACTUCA

THOMAS W. WHITAKER AND ROSS C. THOMPSON

(WITH NINE FIGURES)

This report presents a series of cytological observations of certain interspecific hybrids in the genus *Lactuca*. Previous papers have been concerned with the cytology of the various species, and the genetic aspects of interspecific hybridization in this genus (Whitaker and Jagger 1938, Thompson *et al.*, in press). As far as the available material would permit, the genetic and cytological variability of the species in the genus have been explored with the object of locating suitable material for hybridization with cultivated lettuce (*L. sativa*). Incidentally, observations have been made which we consider contribute to a better understanding of relationships between the several species studied, and we have obtained evidence which throws some light on the evolutionary processes at work within the genus.

The present paper constitutes a joint report of certain work of the past several seasons at the U. S. Horticultural Station, Beltsville, Maryland, and the U. S. Horticultural Field Station, La Jolla, California. It is primarily concerned with the cytological aspects of the problems enumerated above.

## MATERIALS AND METHODS

There were available for study interspecific hybrids of the following species: *Lactuca tatarica* (L.) C. A. Mey. × *L. indica* L.; *L. Raddeana* Maxim. × *L. indica*; *L. sativa* L. × *L. virosa* L.; *L. tatarica* × *L. floridana* (L.) Gaertn. *L. graminifolia* Michx. × *L. indica*; *L. graminifolia* × *L. tatarica*; *L. graminifolia* × *L. virosa*; *L. graminifolia* × *L. floridana*; *L. canadensis* L. × *L. floridana* (for sources of material see Thompson *et al.*, in press). Buds in which the pollen mother cells had initiated meiosis were fixed in an acetic acid–absolute alcohol mixture, and prepared as described elsewhere (see Jagger and Whitaker 1937). The cytological observations have necessarily been limited to those crosses which have produced material suitable for meiotic studies.

## OBSERVATIONS

*L. Raddeana* (9)[1] × *L. indica* (9). Plants obtained from this cross had 9 pairs of chromosomes. There was good synapsis between the two sets of chromosomes at diakinesis (fig. 1). In the course of the work several hundred cells have been observed, but none gave any evidence of irregularities at meiosis. Homology between the two genoms is substantiated by the fact

[1] The figures in parentheses immediately following the specific names are the *n* chromosome numbers of the species.

that the hybrid produced viable seed if harvested at the proper time. It should be pointed out that there were disturbances in the hybrid plants which were not reflected in gross cytological aberrations. For example, the seed produced by the $F_1$ was viable only if harvested prematurely; if allowed to mature the embryos became shriveled, shrunken, and would not germinate.

*L. tatarica* (9) × *L. indica* (9). Hybrids of this mating had 18 somatic chromosomes. Meiotic observations indicated that the two genoms are not completely homologous. Typical cells have 7 bivalents and 4 univalents (fig. 2). There were no instances of multivalent association and 8 was the maximum number of bivalents observed. Chromatin bridges, and unpaired and fragmented chromosomes were observed. As might be expected from these cytological observations, the $F_1$ was entirely sterile.

*L. sativa* (9) × *L. virosa* (9). The $F_1$ plants resulting from this cross had 18 somatic chromosomes. Synapsis between the chromosomes of the genoms was not complete. Frequently the members of a pair, or less frequently, the members of two pairs of bivalents remained unconjugated (fig. 3). Chromatin bridges were of frequent occurrence at IA (fig. 4), interkinesis, and IIA. Numerous fragments were observed; most of them appeared to be acentric and were lost in the cytoplasm. These disturbances at meiosis were sufficient to cause a high degree of sterility. Three per cent of the pollen were viable. However, not a single seed was obtained from the four plants grown to maturity.

*L. tatarica* (9) × *L. floridana* (17). This mating was successful and produced 7 plants, all of which appeared to be interspecific hybrids. It represents a successful case of fertilization in which the female parent, *Lactuca tatarica*, is diploid, and the pollen parent, *L. floridana*, is an amphidiploid.

The $F_1$ plants from this cross had 26 somatic chromosomes. Cells satisfactory for the determination of pairing relationships were observed to have a variable number of bivalents. There were never less than 9 and infrequently as many as 11 bivalents (fig. 5), and occasional trivalents have been observed. In addition to the above features, there were such meiotic irregularities as unpaired chromosomes, chromatin bridges, and fragments.

From the type and quantity of the irregularities found at meiosis, one could safely predict that the $F_1$ plants would be sterile. Such was the case, although they were vigorous vegetatively, and produced an abundance of flowers; there were no viable achenes.

*L. graminifolia* (17) × *L. indica* (9). The $F_1$ plants from this mating had 26 chromosomes in root tip cells. In our observations of meiosis we have counted a maximum of 7 bivalents per cell. The remaining chromosomes are unpaired, and apparently distributed at random during the first division. These cytological irregularities did not result in complete sterility in the $F_1$ plants. A few seed were obtained late in the flowering season.

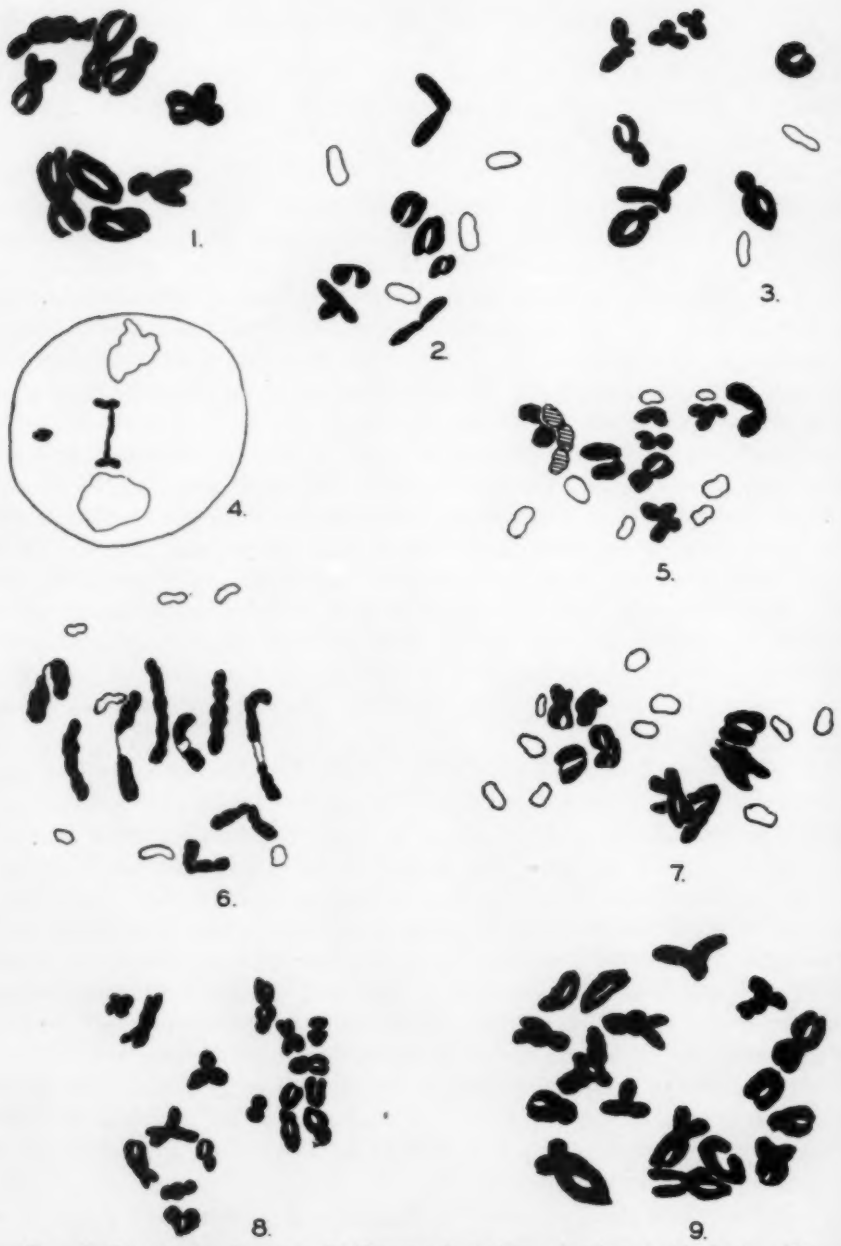


FIG. 1. Diakinesis, *Lactuca Raddeana* × *L. indica*, showing 9 bivalents. FIG. 2. Diakinesis, *L. tatarica* × *L. indica*, showing 7 bivalents, the 4 univalents are drawn in outline. FIG. 3. Diakinesis, *L. sativa* × *L. virosa*; 8 bivalents, 2 univalents. FIG. 4. Late telophase, *L. sativa* × *L. virosa*, showing chromatin bridge and acentric fragment. FIG. 5. Diakinesis, *L. tatarica* × *L. floridana*; 1 trivalent (crosshatched), 8 bivalents, and 7 univalents. FIG. 6. First anaphase, *L. graminifolia* × *L. virosa*; 9 bivalents, 8 univalents. FIG. 7. Diakinesis, *L. graminifolia* × *L. tatarica*; 8 bivalents, 10 univalents. FIG. 8. Diakinesis, *L. graminifolia* × *L. floridana*; 17 bivalents. FIG. 9. Diakinesis, *L. canadensis* × *L. graminifolia*; 17 bivalents.

All figures × 2000, except figure 8 which is × 1500.

*L. graminifolia* (17) × *L. virosa* (9). Although this mating was attempted on numerous occasions only one hybrid plant was obtained. The $F_1$ plant had 26 chromosomes (fig. 6). Pairing was of the *Drosera* type, 9 bivalents plus 8 univalents. There were many irregularities, for example chromatin bridges at IA, and IIA, fragments and even entire chromosomes lost in the cytoplasm. Because of the great number of cytological irregularities we anticipated considerable pollen sterility. Such was the case; only about 9 per cent of the microspores indicated viability by the staining method and no viable seed developed.

*L. graminifolia* (17) × *L. tatarica* (9). The $F_1$ plants of this combination had 26 chromosomes. Meiosis in the hybrid was very irregular. Lagging

TABLE 1

*Summarized cytological observations of species hybrids in Lactuca*

| Species | Chromosome number of $F_1$ | | Per cent viable pollen | Production of seed | Pairing | Cytological irregularities |
|---|---|---|---|---|---|---|
| | n | 2n | | | | |
| *L. Raddeana* (9) × *L. indica* (9) | 9 | 18 | 16.6 | Fair | $9^{II}$ Complete pairing | None |
| *L. tatarica* (9) × *L. indica* (9) | | 18 | — | None | $2–4^{I}$ $7–8^{II}$ | Non-pairing Bridges Fragments |
| *L. sativa* (9) × *L. virosa* (9) | 9 | 18 | 3.0 | None | $1–2^{I}$ $7–8^{II}$ | Non-pairing Bridges |
| *L. tatarica* (9) × *L. floridana* (17) | | 26 | 14.9 | None | $5–7^{I}$ $8–9^{II}$ $1^{III}$ | Univalents Non-pairing Bridges Fragments |
| *L. graminifolia* (17) × *L. indica* (9) | | 26 | 37.9 | Few | $12–14^{I}$ $6–7^{II}$ | Unpaired chromosomes |
| *L. graminifolia* (17) × *L. virosa* (9) | | 26 | 9.0 | 3 seeds | $8^{I}$ $9^{II}$ | Bridges Fragments Unpaired chromosomes |
| *L. graminifolia* (17) × *L. tatarica* (9) | | 26 | — | None | $10^{I}$ $8^{II}$ | Bridges Fragments Unpaired chromosomes |
| *L. graminifolia* (17) × *L. floridana* (17) | 17 | | 90.0 | Good | $17^{II}$ | Occasional fragment and bridge |
| *L. canadensis* (17) × *L. floridana* (17) | 17 | | 9.0 | Fair | $17^{II}$ | None |

chromosomes, unpaired chromosomes, and chromatin bridges were observed. In cells satisfactory for the observation of pairing relationships there were 8 bivalents and 10 unpaired chromosomes (fig. 7). No viable seed was obtained.

*L. graminifolia* (17) × *L. floridana* (17). The $F_1$ plants from this cross between two amphidiploid species had 17 pairs of chromosomes (fig. 8). At diakinesis all chromosomes were associated as bivalents. Multivalent association appeared to be rare or entirely lacking. A few fragments and an occasional chromatin bridge have been observed. Pollen counts indicated between 90 and 95 per cent good pollen. These hybrids were expected to be only slightly less fertile than the parents, and this was actually the case.

*L. canadensis* (17) × *L. floridana* (17). Hybrids from this mating had 17 pairs of chromosomes. The chromosomes were associated as bivalents in all the cells satisfactory for the determination of pairing relationships (fig. 9). A careful search through our material has not revealed a single cytological irregularity.

In table 1 the pertinent observations on the cytology of the $F_1$ plants of various *Lactuca* species described in detail have been summarized.

## DISCUSSION

On the basis of the genetic and cytological evidence the amphidiploid species of *Lactuca* with 17 chromosomes form a closely related group. In fact the evidence suggests the theory that these species are derivatives of a single amphidiploid, i.e., the chromosome complement of the group may be represented by the formula *AABB*, where *A* = the 8 chromosome genom and *B* = the 9 chromosome genom. Facts supporting this statement are as follows.

1. Of the five crosses reported between species with seventeen chromosomes, four have produced $F_1$ plants that were partly or wholly fertile. It is true that there is considerable variation in fertility between the extreme case of *L. graminifolia* × *L. canadensis*[2] in which the $F_1$ is as fertile as either parent and the situation in the hybrids of the mating between *L. canadensis* × *L. floridana* in which only a few seed have been obtained. In only one cross was the $F_1$ sterile (*L. spicata* × *L. graminifolia*). However, the fact that the mating was successful is some indication of relationship.

2. In the $F_1$ plants that have been studied cytologically there is very good synapsis between the genoms of the parental species. Pairing in most crosses takes the form of bivalent association. We have observed very few multivalent configurations in interspecific crosses in this group of species.

3. In at least one cross (*L. canadensis* × *L. graminifolia*) which has been studied intensively it has been possible to demonstrate that specific differ-

[2] Unpublished data in files of the writers.

ences are dependent upon relatively few gene differences (unpublished data on $F_2$, $F_3$, etc., of *L. canadensis* × *L. graminifolia*).

4. The 17-chromosome species of *Lactuca* are indigenous to eastern North America, from Canada to Florida. The fact that they are confined to this limited geographical area would seem to be another indication of close affinity.

It may be argued that the American species of *Lactuca* instead of being amphidiploids have descended from a single 9-chromosome tetraploid *Lactuca* species through the loss of one pair of chromosomes. Circumstantial evidence is against this supposition, because (a) in *L. sativa*, tetraploids produced by colchicine have been extremely sterile, probably as the result of relatively large amount of multivalent association (unpublished data); (b) on the contrary, the American species are very fertile (90–95 per cent good pollen), and any exception to bivalent association is rare.

The possibility that the American amphidiploids are the result of a mating between two 9-paired species and subsequent amphidiploidy with the loss of one pair of chromosomes cannot be entirely dismissed. However, there is no precedent on record for this type of origin, and it is to be expected that the loss of one pair of chromosomes would create serious difficulties interfering with viability.

If full consideration is given to their compatibility relationships and cytological behavior, it is probable that the 17-chromosome amphidiploid species of *Lactuca* have originated from a single occurrence of amphidiploidy. Speciation on the basis of this assumption has taken place through the accumulation of a number of relatively simple gene mutations, with perhaps some structural rearrangement of the chromosomes, since an occasional chromatin bridge has been found in certain crosses.

Having disposed of the question of genetic affinity between the American amphidiploid species of *Lactuca*, it now seems proper to review some of the evidence regarding their origin, that is, to consider which of the 8- or 9-chromosome species are the most likely ancestors of the American amphidiploids. We have no observations that would indicate the 8-chromosome parental species; on the other hand there are some data which suggest the group from which the 9-chromosome species may have come.

On the basis of genetic evidence, and with certain exceptions, the species of *Lactuca* fall into three compatibility groups (Thompson *et al.*, in press). Group I consists of the 9-chromosome species which are more or less closely related to cultivated lettuce (*L. sativa, L. Scariola, L. altaica, L. virosa, L. saligna*). Group II is made up of the following 9-chromosome species: *L. indica, L. Raddeana,* and *L. tatarica.* Group III is composed of the 17-chromosome American species. Of the 14 successful crosses obtained between members of two of these groups (groups II and III), three have been ex-

amined cytologically. In most crosses at least 8 chromosomes from each genom seemed to be homologous. Only one hybrid plant was obtained in hundreds of attempts to hybridize species in group I with the 17-chromosome American species (group III). In this hybrid pairing was of the *Drosera* type, indicating homology between 9 chromosomes of each genom. On the basis of pairing relationships the evidence is not sufficient to be decisive between group I and group II. It is clear from the genetic data that species hybrids are much more easily obtained in matings of the American amphidiploids with group II, and this may mean that the American amphidiploids find their closest affinity with members of this group. We have been informed that the morphological evidence also supports this conclusion, namely, that group III has many more characteristics in common with group II than with group I.[3]

SUMMARY

Observations of the principal details of meiosis in nine $F_1$ hybrids produced from a series of interspecific matings in *Lactuca* are reported. Three of these crosses were between species with 9 pairs of chromosomes, four between species having 9 and 17 pairs of chromosomes, and two between species with 17 chromosomes. Pairing behavior and irregularities are analyzed in these hybrids. It is evident from this analysis that the 9-chromosome species consist of at least two distinct compatibility groups. The 17-chromosome group is very homogenous. It is suggested that the latter group of species has descended from a single amphidiploid or may have resulted from a single occurrence of amphidiploidy.

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[3] Private communication from Dr. G. L. Stebbins, Jr., University of California, Berkeley, California.

# "MULTINUCLEATE" PLANT CELLS

PAUL R. BURKHOLDER AND ILDA MCVEIGH

(WITH ONE FIGURE)

During a study of the growth of some maize lines and hybrids, an apparent multinucleate condition was observed so frequently in cross sections of stems that we were led to investigate the real nature of the condition.

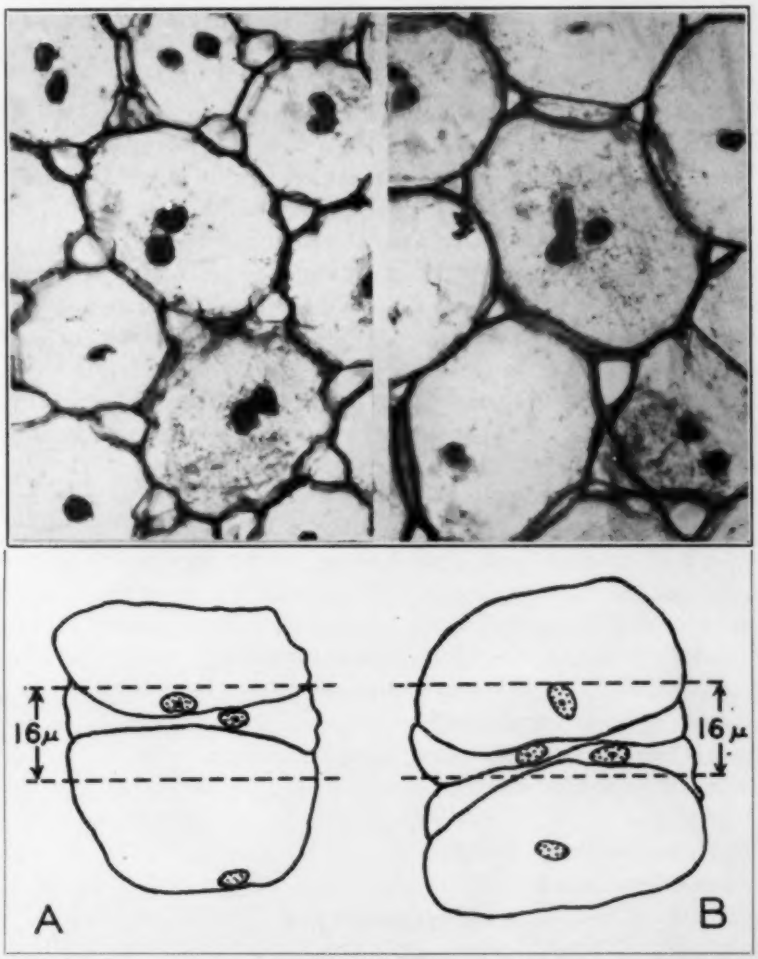


FIG. 1. Above: Transverse sections through internodes of young maize plants showing apparently bi- and trinucleate condition in ground parenchyma. Below: Diagrams of longitudinal sections through internodal parenchyma cells showing in A how cross sections cut 16 microns thick along dotted lines would give an apparently binucleate structure, and in B a trinucleate artifact.

Some workers with whom the phenomenon was discussed expressed the opinion that our inbred lines might be unique in possessing as a general rule multinucleate cells. Others confirmed our observations in the light of their own experiences with maize, and recalled that just one score years ago Beer and Arber (1920) had described multinucleate cells in 177 species representing 60 families of plants. Wipf and Cooper (1940) have since reported binucleate cells occasionally present in the cortical parenchyma of roots of eight species of leguminous plants. Even though a recent paper by Wareham (1936) exposed the fallacy of a so-called "multinucleate phase" in stem development, it appears that many botanists are not yet certain about the condition in parenchyma cells.

Further studies were made on both transverse and longitudinal sections through internodal tissue just above the intercalary meristem of a number of selected lines of maize. Microchemical tests with iodine, crystal violet, ninhydrin, Feulgen reagent, etc., convinced us that the small bodies grouped in the cells were really nuclei, and not starch grains or other debris. In both transverse and longitudinal sections appropriate tests with I–KI and sulphuric acid, and with ruthenium red, permitted clear demonstration of cell walls separating the nuclei, one to each cell. No apparent multinucleate condition was ever noted in longitudinal section. The alleged bi- and trinucleate conditions portrayed in the accompanying photomicrographs (fig. 1) are explained in the lower part of the figure by camera lucida drawings of cells in longisectional view. Many cells of the ground parenchyma adjacent to the meristem are compressed and distorted out of ordinary shape as a result of differential cell expansion in the internode. It is plainly seen how cross sections cut 16 microns thick (dotted lines) can produce multinucleate artifacts. Though the multinucleate condition is known to occur in special instances in the plant kingdom, it is not the true condition in stem parenchyma of maize. This brief note, therefore, substantiates the work of Wareham, who reported that cells of pith and cortex appearing multinucleate in cross section are in reality uninucleate.

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# STUDIES OF PACIFIC ISLAND PLANTS—I

A. C. SMITH

This paper is the first of a series in which it is proposed to discuss new and noteworthy plants from the Pacific area and to revise limited groups of plants as the occasion arises. The geographic area under consideration includes the Polynesian, Micronesian, and Melanesian groups, although the Solomon Islands, being floristically so intimately related to New Guinea, may as a rule be excluded from treatment in this series. A substantial volume of herbarium material has recently been collected in the Pacific, and it seems probable that in the near future additional material will be available for study. The writer has no wish to intrude upon the fields of various students who are occupied with special taxonomic groups or geographic areas in the Pacific, and intends to limit this series to discussions of more or less neglected groups.

## THE PACIFIC SPECIES OF MYRISTICA

In his treatment of the Myristicaceae, Warburg (Nova Acta Acad. Leop.-Carol. **68**: 1–680. *pl. 1–25*. 1897) definitely recognized only three species of *Myristica* in the Pacific, but subsequent collections indicate the existence of eight species, of which one is first described in the present paper. These species occur from Samoa and Tonga westward and are well localized, only one (*M. inutilis*) being known from several major geographic groups. They are distributed as follows: Samoa (2 species), Tonga (1 or possibly 2 species), Fiji (4 or possibly 5 species), New Hebrides (2 species), Carolines (1 species). In the neighboring Solomon Islands, 8 species are known (see A. C. Smith, Jour. Arnold Arb. **22**: 60–80. 1941), and in view of this it seems unlikely that only 2 species actually exist in the New Hebrides; it is to be anticipated that future collectors in this group will discover other species. The distribution of *Myristica* is an interesting illustration of the increasing paucity of species toward the east, as compared with the wealth of species in New Guinea, where at least 34 are now known. The insular species show strong affinities with those of New Guinea, some of them being more closely allied to Papuan relatives than to each other.

Since the Pacific species were originally described, for the greater part, from inadequate material, it seems advisable to include in this treatment descriptions based on the available specimens, except for *M. macrantha*, since the publication of which no additional material has become available. I have been privileged to see the specimens deposited in the following herbaria: Arnold Arboretum (A), Gray Herbarium (GH), New York Botanical Garden (NY), U. S. National Herbarium (US), the place of deposit being indicated by the abbreviations in parentheses. I am indebted to the

directors and curators of the named institutions for the opportunity to examine specimens.

Several characters in the genus *Myristica* have a diagnostic value which has been neglected. The leaf-blades of the Pacific species are often ceriferous beneath, the wax being deposited either as a uniform thin layer or in minute crowded globules. The latter type is illustrated by *M. macrantha* and *M. castaneaefolia*. The type of pubescence on the staminate inflorescence is also a constant character and must be resorted to for the accurate separation of certain species. More obvious characters, such as leaf-proportions and size, venation, type of inflorescence, shape of perianth, and proportions of fruit are also fairly dependable. Leaf-shape, however, is often not a reliable character and hardly seems to justify specific separation unless it is supported by other evidence.

### Key to the Pacific Species

Staminate inflorescence coarsely vermiform, simple or forked near base, the rachises densely cicatricose to base, 4 mm. or more in diameter; perianth cylindric-ellipsoid or campanulate, about twice as long as broad; connective slightly exceeding the anthers; lower surface of leaf-blades farinose- or papillose-ceriferous; Fiji.

Leaf-blades large, 35–60 cm. long, 12–23 cm. broad, rounded to cordate at base; secondary nerves 24–35 per side; rachis about 10 mm. in diameter; perianth about 10 mm. long ..... 1. *M. macrantha.*

Leaf-blades smaller, (10–) 15–32 cm. long, (4.5–) 6–14 cm. broad, obtuse to rounded at base; secondary nerves 17–27 per side; rachis 4–5 mm. in diameter; perianth 5–7 mm. long ..... 2. *M. castaneaefolia.*

Staminate inflorescence comparatively slender, the rachises rarely exceeding 4 mm. in diameter, the primary (and often secondary) peduncle usually obvious; lower surface of leaf-blades often ceriferous but smoothly so (rarely in no. 5 farinose-ceriferous).

Leaf-blades persistently pale-tomentellous beneath; staminate perianth urceolate-cylindric, at least twice as long as broad; androecium-stalk nearly as long as anthers, the connective obviously produced; fruit oblong-ellipsoid, much longer than broad; Samoa to New Hebrides (and Solomons) ..... 3. *M. inutilis.*

Leaf-blades glabrous, at least at maturity; staminate perianth 1–2 times as long as broad; androecium-stalk conspicuously shorter than anthers, the connective not (or faintly) produced.

Staminate inflorescence simple, the peduncle 1–1.5 mm. in diameter, swollen distally, the perianth campanulate, nearly twice as long as broad; leaf-blades comparatively small (6–15 cm. long, 2.5–6.5 cm. broad), inconspicuously ceriferous and concolorous, the secondary nerves inconspicuous; Fiji ..... 4. *M. chartacea.*

Staminate inflorescence usually obviously forked, the peduncle 1.5–4 mm. in diameter, the rachises usually elongate, the perianth subglobose or broadly campanulate, nearly as broad as long; leaf-blades larger (12–35 (–45) cm. long, (4–) 6–16 cm. broad), conspicuously ceriferous and usually glaucous beneath, the secondary nerves obvious, strongly prominulous beneath.

Leaf-blades about twice as long as broad, the secondary nerves comparatively crowded; staminate inflorescence tomentellous (hairs several-celled, the lower cells conspicuously trans-

versely fusiform); anthers 11–18; fruit ellipsoid (37–50 mm. long, 23–30 mm. broad); Carolines ..... 5. *M. insularis.*

Leaf-blades usually 3–4 times as long as broad, the secondary nerves comparatively spaced; anthers 7–11.

Staminate inflorescence strigose (hairs simple or obscurely plumulose near base); fruit broadly ellipsoid or subglobose (28–40 mm. long, 24–30 mm. broad); Samoa and Tonga ..... 6. *M. hypargyraea.*

Staminate inflorescence tomentellous (hairs branched from or near base, tangled); fruit oblong-ellipsoid, conspicuously longer than broad (37–50 mm. long, 23–28 mm. broadly); Fiji ..... 7. *M. Gillespieana.*

Species of this relationship; leaf-blades chartaceous, obscurely ceriferous; inflorescence lacking; fruit essentially subglobose (27–32 mm. in diameter); New Hebrides 8. *M. Guillauminiana.*

1. MYRISTICA MACRANTHA A. C. Smith, Bishop Mus. Bull. **141**: 67. *f. 33.* 1936.

The original description was based on the material cited below and therefore does not need amplification.

*Distribution:* Fiji, known only from Vanua Levu at elevations up to 700 m.

FIJI—VANUA LEVU: Mbua, lower Wainunu River valley, *Smith 1719* (GH, NY—TYPE, US); Thakaundrove, southwestern slope of Mount Mbatini, *Smith 613* (GH, NY, US).

*Common name: Male wangga.*

The species is closely related only to *M. castaneaefolia,* being readily separable by the great size of all its parts. While this difference may not be very dependable as regards foliage, it is very striking in a comparison of the staminate inflorescences and perianths.

2. MYRISTICA CASTANEAEFOLIA A. Gray, Bot. U. S. Expl. Exped. **1**: 32. 1854; A. DC. in DC. Prodr. **14**: 193. 1856; Seem. Fl. Vit. 205. 1867; Warb. Nova Acta Acad. Leop.-Carol. **68**: 492 (as *M. castaneifolia*). *pl. 18.* 1897.

Tree to 18 m. high, the vegetative parts essentially glabrous throughout, the branchlets stout, subterete, soon cinereous and rugulose; petioles stout (1.5–5 mm. diam.), 12–35 mm. long, brownish or nigrescent, shallowly canaliculate; leaf-blades chartaceous or thin-coriaceous, oblong-elliptic, (10–) 15–32 cm. long, (4.5–) 6–14 cm. broad, obtuse or rounded at base, obtuse or obtusely cuspidate at apex, slightly reflexed at margins, dark brown above, glaucous beneath and farinose- or papillose-ceriferous (rarely smoothly ceriferous when young), the costa conspicuously raised above, prominent beneath, the secondary nerves 17–27 per side, spreading, straight, anastomosing near margins, impressed or faintly raised above, prominent beneath, the veinlets obscure, sometimes faintly impressed above and prominulous beneath; staminate inflorescence coarsely vermiform, up to 25 mm. long, usually forked near base, the peduncle stout (about 4 mm. in diameter), 1–6 mm. long, the secondary peduncles none, the rachises stout (4–5 mm. in diameter), very densely cicatricose, pubescent distally, the bracts ovate, minute, caducous; flowers aggregated toward ends of rachises, densely fer-

ruginous-strigose or -sericeous (hairs about 0.7 mm. long, simple or obscurely plumulose near base), the pedicels very short, the bracteole deltoid-ovate, 1–1.5 mm. long, subacute; perianth thick-carnose, cylindric-ellipsoid, 5–7 mm. long, 2.5–3.5 mm. in diameter, the lobes 3, deltoid-oblong, subacute, about 1 mm. long; androecium 4–5 mm. long, the stalk stout, 1–1.5 mm. long, shortly castaneous-strigose near base, the anthers 8–10, 2.5–3.5 mm. long, the connective stout, obtuse and slightly (0.2–0.5 mm.) projecting at apex; fruit subsessile at ends of short inflorescences, ellipsoid or ovoid-ellipsoid, often inaequilateral and obscurely carinate, 28–38 mm. long, 18–24 mm. broad, obtuse to truncate at base, rounded and minutely apiculate at apex, densely ferruginous-tomentellous, the pericarp 2–3 mm. thick, the aril copiously laciniate.

*Distribution:* Fiji, apparently fairly abundant at elevations up to 900 m. FIJI: *Horne 205* (GH), *243* (GH). VITI LEVU: Tholo North, Nandarivatu, *Gillespie 4312* (NY, US); Namosi, Mount Naitarandamu, *Gillespie 3357* (NY); Naitasiri, Tamavua woods, near Suva, *Gillespie 2032* (NY), *2056* (US). OVALAU: *U. S. Expl. Exped.* (GH—TYPE, NY, US). VANUA LEVU: Mbua, Seatovo Range, *Smith 1537* (GH, NY, US); lower Wainunu River valley, *Smith 1745* (GH, NY, US).

*Common names: Male, Male ndina.*

The specimens from Vanua Levu have slightly smaller leaves than the others and do not show such a pronounced farinose-ceriferous lower surface of the leaf-blades, but there seems no doubt of their place here. I erroneously mentioned (Bishop Mus. Bull. **141**: 66. 1936) one of these specimens as *M. hypargyraea*, a species not yet known from Fiji.

3. MYRISTICA INUTILIS Rich ex A. Gray, Bot. U. S. Expl. Exped. **1**: 34. 1854; A. DC. in DC. Prodr. **14**: 191. 1856; Warb. Nova Acta Acad. Leop.-Carol. **68**: 481. *pl. 18.* 1897; Guillaumin, Jour. Arnold Arb. **14**: 59. 1933; Christophersen, Bishop Mus. Bull. **128**: 87. 1935; A. C. Smith, Jour. Arnold Arb. **22**: 74. 1941.

Slender tree, up to 20 m. high, the young parts ferruginous-sericeous, the branchlets slender (1–2.5 mm. in diameter distally), subterete, pale brown, at length cinereous, rugose, lenticellate; petioles slender (1–3 mm. in diameter), 10–28 mm. long, pale brown or nigrescent, shallowly canaliculate; leaf-blades papyraceous or chartaceous, oblong- or obovate-elliptic, 12–23 cm. long, 4–8.5 cm. broad, acute to rounded at base, gradually acuminate or cuspidate at apex, glabrous and dark olivaceous above, paler and persistently pale tomentellous beneath, the costa slightly raised above, prominent beneath, the secondary nerves 14–22 per side, erecto-patent, slightly arcuate, obscurely anastomosing, essentially plane or slightly impressed above, prominulous beneath, the veinlets usually obscure; staminate inflorescence axillary or arising from defoliate branchlets, up to 12 mm. long, usually forked near base, rarely simple, the peduncle 1.5–3 mm. in diameter, 1–4 mm. long, the secondary peduncles very short or essentially none, the rachises 2.5–3 mm. in diameter, cicatricose nearly to base, pubescent distally, the bracts ovate, minute, caducous; flowers crowded distally on rachises, pale ferruginous-tomentellous (hairs usually 3–6-branched from base, 0.2–0.4 mm. long, rarely simple), the pedicels slender, up to 4 mm. long, the bracteole submembranous, ovate-deltoid or oblong, obtuse, 1.5–4

mm. long, 1–2 mm. broad; perianth urceolate-cylindric, 4–5 mm. long, 1.5–2.5 mm. in diameter, the lobes 3, deltoid, subacute, 1–1.5 mm. long and broad; androecium 3.5–4.5 mm. long, the stalk slender, 1.5–2 mm. long, faintly stramineous-strigose near base, the anthers 8–11, 1.7–2.5 mm. long, the connective slender, obtuse, usually obviously exceeding the anthers; fruits solitary or paired, usually axillary, the peduncle 4–6 mm. long, 2.5–4 mm. in diameter, swollen distally; fruit oblong-ellipsoid, 23–40 mm. long, 15–23 mm. broad, obtuse at base, obtuse and minutely apiculate at apex, persistently pale tomentellous, the pericarp about 1 mm. thick, the aril finely laciniate, the seed oblong-ellipsoid, up to 32 mm. long and 17 mm. broad.

*Distribution:* Samoa (and perhaps Tonga) and the New Hebrides, at elevations up to 500 m.; also known from the Solomons.

NEW HEBRIDES—EFATE: Undine Bay, *Kajewski 220* (A). TANNA: Lenakel, *Kajewski 44* (A). ANEITYUM: Anelgauhat Bay, *Kajewski 757* (A). SAMOA: U. S. Expl. Exped. (GH—TYPE, NY, US); *Whitmee* (GH); *Graeffe 79* (GH); *Vaupel 642* (US); *Kuntze 21084* (NY). TAU: near Faleasao, *Garber 609* (NY). TUTUILA: near Pango Pango, *Kuntze 23015* (NY); *Potts 1* (US); above Naval Station, *Christophersen 984* (US), *995* (NY). SAVAII: Salailua, *Christophersen 2612* (US); Tanga, *Christophersen 2338* (NY); near Samalaeulu, *Christophersen 3473* (NY, US). TONGA (?): *U. S. Expl. Exped.* (US).

*Common name: Atone, Atoge* (in Samoa).

Although one of the Exploring Expedition specimens, as cited, bears the inscription "Tonga," and although the species is mentioned in the original publication as being from "Tutuila, Savaii, and Tongatabu," it may be advisable not to record this species definitely from Tonga until additional and more accurately labeled specimens are available.

The species is a very distinct one and has no close relatives among Pacific species, being apparently of the alliance of the Papuan *M. Buchneriana* Warb. The New Hebrides specimens have the fruits proportionately slightly broader, but this small difference hardly seems to distinguish them specifically from the Samoan specimens. The absence of *M. inutilis* from Fiji is noteworthy and is possibly to be explained by the inadequacy of our material from that group. Christophersen cites several Samoan specimens not available to me, the species being fairly abundant in that group.

4. MYRISTICA CHARTACEA Gillespie, Bishop Mus. Bull. **83**: 5. *f. 2.* 1931; A. C. Smith, Bishop Mus. Bull. **141**: 66. 1936.

Slender tree, up to 15 m. high, the young parts tomentellous, the branchlets subterete, very slender (1–3 mm. in diameter), pale brown, at length cinereous and rugose; petioles slender (1–2 mm. in diameter), 10–26 mm. long, pale brown, shallowly canaliculate; leaf-blades chartaceous, oblong-elliptic, 6–15 cm. long, 2.5–6.5 cm. broad, rounded to acute at base, rounded to obtusely short-acuminate at apex, slightly recurved at margins, olivaceous and concolorous, obscurely and smoothly ceriferous beneath, the costa conspicuously raised above, prominent beneath, the secondary nerves 17–22 per side, erecto-patent, straight, obsoletely anastomosing, essentially plane or faintly impressed above, plane or prominulous beneath, the veinlets obscure, often slightly impressed above; staminate inflorescence axillary or arising

from defoliate branchlets, up to 10 mm. long, simple, the peduncle 1–1.5 mm. in diameter, 2–4 mm. long, swollen distally, faintly stramineous-strigose, the bracts broadly ovate, minute; flowers fasciculate at apex of peduncle or crowded on a very short rachis, ferruginous-tomentellous (hairs branched, about 0.3 mm. long), the pedicels up to 1.5 mm. long, the bracteole submembranous, ovate, about 4 mm. long and broad, obtuse; perianth thin-carnose, campanulate, 4.5–5 mm. long, 2.5–3 mm. broad, the lobes 3, deltoid, about 1.5 by 2.5 mm.; androecium stout, carnose, 3.5–4 mm. long, the stalk glabrous, about 1 mm. long, the anthers 6 or 7, 2.5–3 mm. long, free distally and often slightly exceeding the apically convex connective; pistillate inflorescences essentially similar to staminate; perianth urceolate, to 4 mm. in diameter; ovary ovoid, about 2.5 mm. in diameter at anthesis, densely ferruginous-strigose, the stigma deeply cleft; fruits usually solitary, often subsessile, the peduncle up to 10 mm. long and 4 mm. in diameter; fruit ovoid-ellipsoid, 27–50 mm. long, 17–30 mm. in diameter, usually truncate at base, rounded and minutely apiculate at apex, persistently tomentellous, the pericarp rugose, thin (1–2 mm. thick), the aril laciniate into broad lobes, the areoles large, the seed oblong-ellipsoid, up to 40 by 24 mm.

*Distribution:* Fiji, at elevations between 200 and 900 m.

FIJI: *Seeman 7* (GH). VITI LEVU: *Horne 966* (GH). VANUA LEVU: Thakaundrove, Yanawai River region, Mount Kasi, *Smith 1825* (GH, NY, US); southern slope of Korotini Range, *Smith 501* (GH, NY, US). MOALA: near Naroi, *Smith 1316* (GH, NY, US), *1319* (GH, NY, US).

*Common names: Male, Wale.*

The type is *Gillespie 4206*, from Nandarivatu, Tholo North, Viti Levu; additional specimens are cited by Gillespie from Namosi and Naitasiri provinces, Viti Levu. The species is readily recognized by its small leaves with inconspicuous nerves and is perhaps most closely related to *M. Gillespieana;* it can be at once distinguished from *M. castaneaefolia* by its lack of the powdery wax characteristic of the under surface of leaf-blades of that species, as well as by obvious floral and other foliage differences.

5. MYRISTICA INSULARIS Kanehira, Fl. Micrones. 115. *f. 35.* 1933; Bot. Mag. Tokyo **47**: 671. 1933.

Tree, up to 10 m. high, the young parts ferruginous-sericeous, soon glabrescent, the branchlets stout (3–5 mm. in diameter distally), rugose, subterete, brown or cinereous; petioles stout (3–4 mm. in diameter), 10–40 mm. long, brownish or nigrescent, conspicuously canaliculate; leaf-blades thin-coriaceous, oblong- to obovate-elliptic, 13–30 cm. long, 5.5–15 cm. broad, rounded to obtuse at base, obtuse at apex, often conspicuously recurved at margins, dark olivaceous above, glaucous and smoothly (rarely inconspicuously farinose-) ceriferous beneath, sometimes sparsely tomentellous beneath when young, soon glabrescent, the costa conspicuously elevated above, prominent beneath, the secondary nerves 16–25 per side, erecto-patent, anastomosing toward margins, usually impressed or occasionally prominulous above, prominent beneath, the veinlets usually impressed above and plane beneath, occasionally faintly prominulous on both sides; staminate inflorescence supra-axillary or arising from defoliate branchlets, 20–40 mm. long, forked, the peduncle 2–3 mm. in diameter, 10–20 mm. long, closely ferruginous-strigose, the secondary peduncles up to 3 mm. long, sometimes

essentially none, the rachises, about 3 mm. in diameter, elongate, cicatricose, ferruginous-strigose, the bracts caducous; flowers crowded near apices of rachises, ferruginous-tomentellous (hairs 0.3–0.6 mm. long, several-celled, the lower cells conspicuously transversely fusiform, the apical cell often elongate), the pedicels 2–4 mm. long at anthesis, stout, the bracteole submembranous, broadly ovate to deltoid, 2–3 mm. long, 4–5 mm. broad, obtuse; perianth campanulate, 4–5 mm. long and broad, the lobes 3, deltoid, obtuse, about 1.5 by 3 mm.; androecium stout, 3.5–4.5 mm. long, the stalk 1–1.5 mm. long, sparsely and obscurely stramineous-strigose, the anthers 11–18, 2–3 mm. long, faintly exceeded by the obtuse connective; fruits apparently usually solitary, the peduncle 3–7 mm. long, stout (4–6 mm. in diameter); fruit oblong-ellipsoid, 37–50 mm. long, 23–30 mm. broad, rounded or obtuse at both ends and inaequilaterally apiculate at apex, densely ferruginous-tomentellous, the pericarp brittle, about 1 mm. thick, the aril laciniate into thin lobes, the areoles often large, the seed oblong-ellipsoid, conspicuously marked by the impressions of the aril.

*Distribution:* Caroline Islands, at elevations up to 300 m.

CAROLINE ISLANDS—TÔDAIYAMA, near Korror: *Kanehira 1865* (NY, type coll.), *2024* (NY, US). BABELDAOB: Galdok, *Kanehira & Hatusima 5009* (NY). PONAPE: *Kanehira 727* (A, NY), *763* (NY), *1512* (NY), *1545* (NY, US).

*Common name: Karala* (Ponape).

Some of the above-cited specimens have been mentioned by Kanehira (Bot. Mag. Tokyo **45**: 280. 1931; Fl Micrones. 113. *f. 34.* 1933) as *M. hypargyraea*, but I believe them to be all conspecific and to represent an endemic species. *M. insularis* may be separated from its Samoan and Fijian relatives (*M. hypargyraea* and *M. Gillespieana*) by the slight differences in foliage mentioned in my key, and more accurately by the difference in type of pubescence on the staminate inflorescence. This latter character, although it can be observed only with a magnification of 25 times or more, is very constant and is probably the best means of distinguishing *M. insularis*.

6. MYRISTICA HYPARGYRAEA A. Gray, Bot. U. S. Expl. Exped. **1**: 33. 1854; A. DC. in DC. Prodr. **14**: 194. 1856; Warb. Nova Acta Acad. Leop.-Carol. **68**: 479. *pl. 18.* 1879; Christophersen, Bishop Mus. Bull. **128**: 86. 1935.

Tree to 10 m. high or more, the young parts faintly puberulent, soon glabrescent, the branchlets subterete, stout (2–5 mm. in diameter distally), pale brown to cinereous, at length lenticellate and rugose; petioles rugose, 1.5–5 mm. in diameter, 15–35 (–43) mm. long, shallowly canaliculate; leaf-blades thin-coriaceous, oblong or elliptic- or obovate-oblong, 12–35 (–45) cm. long, 4.5–14 cm. broad, rounded to subacute at base, cuspidate or obtuse at apex, slightly recurved at margins, olivaceous and shining above, somewhat glaucous and smoothly ceriferous beneath, the costa sharply raised above, prominent beneath, the secondary nerves 15–25 per side, spreading, straight, ascending and anastomosing toward margins, slightly impressed or faintly elevated above, strongly prominulous or prominent beneath, the veinlets usually obscure, sometimes faintly prominulous or impressed on both surfaces; staminate inflorescences supra-axillary or arising from defoliate branchlets, 10–40 mm. long, forked or occasionally trifid (sometimes

simple, at least when young) the peduncle 1.5–3 mm. in diameter, 7–20 mm. long, often persistently strigose, the secondary peduncles up to 5 mm. long, sometimes essentially none, the rachises 3–4 mm. in diameter, cicatricose, puberulent distally, the bracts caducous; flowers crowded distally on rachises, ferruginous-strigose (hairs 0.3–0.7 mm. long, simple or obscurely plumulose near base), the pedicels to 7 mm. long, the bracteole carnose, suborbicular, 3–4 mm. long, 4–5 mm. broad, rounded at apex; perianth carnose, campanulate, at anthesis 5–6 mm. long and broad, the lobes 3, oblong-deltoid, 2.5–3 mm. long, 3–5 mm. broad, obtuse; androecium 2–3.5 mm. long, the stalk stout, glabrous, 0.5–1.3 mm. long, the anthers 7–10, 1.5–2.3 mm. long, faintly exceeding the often apically depressed connective; fruits usually solitary, the peduncle (combined with pedicel) up to 17 mm. long and 4–5 mm. in diameter, often persistently puberulent; fruit broadly ellipsoid or nearly subglobose, 28–40 mm. long, 24–30 mm. broad, rounded at both ends and usually apiculate at apex, persistently strigose or conspicuously tomentellous, the pericarp 1–2 mm. thick, the aril copiously laciniate into narrow lobes, the seed 21–34 mm. long, 18–25 mm. broad.

*Distribution:* Samoa and Tonga, at elevations up to 700 m.

SAMOA: *U. S. Expl. Exped.* (GH—TYPE, NY, US); *Whitmee* (GH); *Kuntze* (NY); *Horne 10* (GH); *Vaupel 300* (NY). TUTUILA: above Naval Station, *Christophersen 996* (NY). UPOLU: Apolau, *Reinecke 133* (US); above Malololelei, *Christophersen 150* (NY, US), *314* (NY). SAVAII: near Samalaeulu, *Christophersen 3474b* (NY). TONGA—TONGATABU: Mua, *Setchell & Parks 15275* (GH, US). EUA: *Parks 16160* (GH, NY, US).

*Common names: Atone, Atone 'ulu* (Samoa).

Christophersen has cited several additional Samoan specimens. The original collection is mentioned as from "Tutuila and Savaii" and the species is said to be also from Tongatabu, but I have seen no Exploring Expedition material of the species from Tonga.

7. MYRISTICA GILLESPIEANA A. C. Smith, Bishop Mus. Bull. **141**: 67. *f. 32.* 1936.

Tree to 20 m. high, the young parts soon glabrous, the branchlets terete, 2–4 mm. in diameter distally, brownish, at length cinereous, rugose, and lenticellate; petioles brownish or nigrescent, 2.5–4 mm. in diameter, 10–50 mm. long, shallowly canaliculate; leaf-blades thin-coriaceous, oblong or obovate- or elliptic-oblong, 16–32 cm. long, 5–11 cm. broad, rounded or obtuse or faintly subcordate at base, obtuse or obtusely cuspidate at apex, slightly recurved at margins, olivaceous or dark green above, somewhat glaucous and smoothly ceriferous beneath, the costa slightly elevated to prominent above, prominent beneath, the secondary nerves 18–26 per side, erecto-patent, straight, inconspicuously anastomosing, faintly impressed or essentially plane above, sharply raised beneath, the veinlets often obscure, faintly prominulous or slightly impressed above, immersed or faintly prominulous beneath; staminate inflorescences supra-axillary or arising from defoliate branchlets, (6–) 10–30 mm. long, forked or occasionally trifid, the peduncle 3–4 mm. in diameter, up to 10 mm. long, glabrous, the secondary peduncles up to 8 mm. long, sometimes essentially none, the rachises 3–4 (–5) mm. in diameter, cicatricose, puberulent distally, the bracts broadly ovate, small, caducous; flowers crowded toward apices of rachises, densely ferruginous-tomentellous (hairs branched from or near base, tangled, not exceeding 0.5 mm. in length), the

pedicels up to 6 mm. long, the bracteole ovate-deltoid, 3–5 mm. long, 4–6 mm. broad; perianth carnose, subglobose or broadly campanulate, 3.5–6 mm. long and broad, deeply cleft, the lobes 3, deltoid, 1.5–4 mm. long and broad; androecium 2–4 mm. long, the stalk stout, glabrous or obscurely stramineous-strigillose, 0.5–1.3 mm. long, the anthers 8–12, 1.5–2.7 mm. long, slightly exceeding or essentially equalling the obtuse or apically depressed connective; pistillate inflorescence essentially similar, the ovary ovoid, at anthesis about 3 mm. long and in diameter, densely ferruginous-strigose (hairs about 0.5 mm. long), the stigma glabrous, inconspicuously cleft; fruits solitary or paired (peduncle up to 8 mm. long and 10 mm. in diameter, the pedicel also stout, up to 15 mm. long), oblong-ellipsoid, 37–50 mm. long, 23–30 mm. broad, rounded to acute or gradually attenuate at base, rounded to subacute and apiculate at apex, closely ferruginous-tomentellous, the pericarp 1.5–3 mm. thick, the aril finely laciniate, the seed 34–40 mm. long, 20–23 mm. broad.

*Distribution:* Fiji, at elevations up to 500 m.

FIJI—VITI LEVU: *Seemann 6* (GH). KANDAVU: above Namalata and Ngaloa Bays, *Smith 124* (GH, NY, US). MOTURIKI: *Storck 866* (GH). VANUA LEVU: Mbua, upper Ndama River valley, *Smith 1597* (GH, NY, US). KORO: *Smith 946* (GH, NY—TYPE, US), *1048* (GH, NY, US). MALATTA, south of Vanua Mbalavu: *Smith 1457* (GH, NY, US).

*Common name: Male.*

In first describing this species, I cited only the type and the Seemann specimen, inadvertently referring the others to *M. castaneaefolia;* subsequent study indicates that the two species are not closely related and that they can be readily distinguished even when sterile by examining the waxy lower surface of leaf-blades. *M. Gillespieana* is less readily separated from the Samoan *M. hypargyraea*, doubtless its closest relative, but the characters of inflorescence-tomentum and fruit-shape mentioned in the key seem sufficiently constant to permit the two species to be retained.

8. **Myristica Guillauminiana** A. C. Smith, sp. nov.

Arbor ad 25 m. alta praeter partes novellas puberulas et fructus glabra, ramulis subteretibus rugosis crassis (apices versus 3–5 mm. diametro) demum conspicue lenticellatis; petiolis rugosis 1.5–2.5 mm. diametro 15–30 mm. longis canaliculatis; laminis chartaceis elliptico- vel obovato-oblongis, 16–24 cm. longis, 5–9.5 cm. latis, basi obtusis et in petiolum inconspicue decurrentibus, apice obtusis vel obtuse cuspidatis, margine paullo undulato-recurvatis, supra olivaceis subnitentibus, plus minusve concoloribus et subtus inconspicue et leviter ceriferis, costa utrinque prominente, nervis secundariis utrinsecus 15–18 erecto-patentibus paullo curvatis inconspicue anastomosantibus supra leviter impressis vel prominulis subtus valde elevatis, venulis inconspicuis supra paullo impressis subtus inconspicue prominulis; fructibus supra-axillaribus vel e ramulis defoliatis orientibus solitariis vel binis, pedunculis crassis (5–6 mm. diametro) brevibus vel subnullis, pedicellis crassis ad 5 mm. longis primo puberulis mox glabris; fructibus subglobosis 27–32 mm. (ad 45 mm. ex Kajewski) diametro manifeste circumcarinatis, utrinque rotundatis et apice conspicue apiculatis, pericarpio tenui ad 1 mm. crasso extra pilis brevibus plumulosis implicatis densissime ferrugineo-tomentello, arillo in lacinias paucas latas fisso, areolis magnis, semine subgloboso.

*Distribution:* New Hebrides, known only from the type collection.

NEW HEBRIDES—VANUA LAVA, Banks group: *Kajewski 422* (A—TYPE, NY), common in rain-forest near sea-level (large tree 25 m. high; fruit brown, up to 4.5 cm. long and 4 cm. in diameter).

It is a pleasure to name this species in honor of Prof. A. Guillaumin, in recognition of his valuable contributions to our knowledge of the flora of the New Hebrides. The specimen was mentioned by Guillaumin (Jour. Arnold Arb. **13**: 83. 1932) as *Myristica* aff. *Hollrungii* Warb., and indeed this may be its closest relative. *Myristica Hollrungii*, however, in addition to having leaf-blades which are on the average larger and slightly thicker in texture than those of the new species, has fruits which are more elongate in proportion and glabrous at maturity. *Myristica Guillauminiana* is readily separated from *M. Gillespieana* by the proportions of its fruits and from *M. hypargyraea* by its thinner leaf-blades which lack the conspicuous glaucous coat of wax of the Samoan species.

### INSUFFICIENTLY KNOWN SPECIES

MYRISTICA GRANDIFOLIA A. DC. in DC. Prodr. **14**: 194. 1856; Seem. Fl. Vit. 205. 1867.

*Myristica macrophylla* A. Gray, Bot. U. S. Expl. Exped. **1**: 33. 1854; non Roxb. (1832); non Benth. (1853).

*Myristica grandifolia* is based on a single sterile specimen from Ovalau, Fiji (*U. S. Expl. Exped.*, US, TYPE), which is to be compared with *M. castaneaefolia* and *M. macrantha*. From the first of these it differs by its tremendous leaves, which are reported to attain a length of 2½ feet. Nevertheless, it is entirely possible that leaves from a young shoot of *M. castaneaefolia* are represented; Warburg (Nova Acta Acad. Leop.-Carol. **68**: 494. 1897) has discussed the specimen as a dubious synonym of this species. *M. grandifolia* differs from *M. macrantha* by having its leaf-blades tapering to an acute base, and I doubt if it can be referred to this species. Its true position can be established only by additional field work, which may prove that a third species of this alliance is represented.

MYRISTICA HORNEI Warb. Nova Acta Acad. Leop.-Carol. **68**: 107, nomen. 1897.

This name is merely listed with a question by Warburg and apparently is not again mentioned in his work. It is likely that he had in mind, as the basis of the name, *Horne 966*, which was later (p. 494) briefly characterized as a second species from Fiji (in addition to *M. castaneaefolia*). The Horne specimen is referable to *M. chartacea*, but since Warburg has not definitely tied his name to this specimen, we may certainly continue to use Gillespie's specific epithet and to treat *M. Hornei* as a nomen nudum.

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JAMAICA PLAIN, MASSACHUSETTS

# FOREST REPLACEMENT RATES IN THE COLORADO HEADWATERS AREA[1]

RONALD L. IVES

Field studies in the Colorado Headwaters area, largely in Grand County, Colorado, near latitude 40°, between the elevations of 8,000 and 11,000 feet, augmented by studies of numerous photographic collections, including those of the U. S. Geological Survey and of the University of Colorado Museum, supply quantitative data concerning replacement of burned-off evergreen forest in this high-altitude environment.

The normal evergreen forest in parts of this area was burned off during Indian troubles in 1862 and 1863; this created large areas of fire slash, in a few of which even the soil was destroyed down to bedrock.

Photographs taken in 1872 by William H. Jackson, official photographer for the Hayden expedition,[2] show many of the fire-damaged trees dead and barkless, grass growing in the charred soil, and brushy growths springing up in especially favored locations.

Between 1872 and 1887–8, when the next series of clear photographs was taken,[3] many of the fire-killed trees had fallen; aspen, the normal replacement growth, was well established, and in a few places overtopped the dense brush that had grown up since 1872.

Since 1898, the area has been photographed many times.[4] Pictures taken during the decade of 1898–1908 show a rapid crowding out of brushy growths by aspen, which reached its maximum height and density, in normal areas, by about 1915. Small evergreens are detectable in several photographs taken as early as 1900, and by 1920 evergreens just overtopped aspens in many locations.

Many of these old fire scars, in photographs taken about 1920, show an aspen forest containing patches (up to 40 per cent) of evergreen. By 1935 evergreen definitely predominated (60 + %), and by 1940 the forest in these old fire scars was almost entirely evergreen, with aspen present only where evergreen growth was inhibited, as by solifluction or rapid sedimentation.

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[1] Field studies supported in part by grants from the Penrose Fund of the American Philosophical Society.

[2] Many of these photographs, in excellent condition, are in the files of the U. S. Geological Survey. Others are in the Ford Museum, at Dearborn, Michigan. An extensive collection of William H. Jackson's prints is in the Denver (Colo.) Public Library. Additional data may be found in "Time Exposure," by William H. Jackson (Putnam, New York, 1940).

[3] These photographs, in private collections at Grand Lake, are dated, but not signed.

[4] These data are from the Brackett, Henderson, and Tangen photographs, most of which are now in the University of Colorado Museum.

Cuttings from several evergreens in these old fire scars indicate ages exceeding 40 years.

Although areas in which the evergreen forest was destroyed in the 1860's now again contain evergreen forest, they are still easily recognizable, for "replacement" evergreen forest is composed of younger trees than the "residual forest." In consequence, the old scars are visible as areas of definitely smaller trees. Trees in the residual forest have ages up to 350 years, as determined by ring counts.

From these data, the following replacement rates, in terms of years since forest destruction, are indicated for this area:

| | | |
|---|---|---|
| Maximum brush growth | 25 | years |
| Maximum aspen growth | 40 | years |
| Aspen largely eliminated | 65 | years |
| Complete elimination of scar | 300+ | years (estimated) |

Where water supply is abnormal, whether too great or too slight, exposure is great, or soil is unstable, rates will be slower. So far as can be determined from this evidence, which is based on foliage area, rather than on "tree counts," transitions from one state to another are rather abrupt, each crowded-out community disappearing rather rapidly once it is overtopped by the next member of the replacement sequence.

Further studies of this type, in areas where old photographs are available, such as those deforested during the Civil War, should produce additional data concerning replacement rates.

Photographs now being taken by the U. S. Forest Service and other organizations should supply much similar data in the future, particularly when the dates and locations are plainly marked on the negatives.

BOULDER, COLORADO

# CACTI OF THE CANYON OF THE COLORADO RIVER AND TRIBUTARIES[1]

ELZADA U. CLOVER AND LOIS JOTTER

(WITH EIGHT FIGURES AND A MAP)[2]

The Canyon of the Colorado has been known to geologists since the time of Ives and Powell, but, except for a restricted section included in the Grand Canyon National Park, little or no botanical work has been attempted before the present investigations.

This paper is the result of field studies, the first made during the summer of 1938 on the Nevills Colorado River Expedition. The party traveled in small boats from Greenriver, Utah, to Boulder Dam, Nevada. The manner of travel made it impossible to collect cacti in quantity, since they are bulky whether obtained living or dry. All species observed were collected, some several times, and detailed notes were taken throughout the canyon.

Two stops for supplies were made during the 666 mile journey, at Lee's Ferry and at Grand Canyon. Here plants, which had been carried in closed hatches, were sent back to the University of Michigan Botanical Gardens.

A second expedition was made by the senior author the following summer. This included trips on horseback and on foot down the Kaibab Trail in Grand Canyon to the Colorado; into the Rainbow Bridge area, southern Utah; and Havasupai Canyon, a tributary of the Colorado. The Park Service at Lake Mead made it possible to go to the head of the lake. Collections were made at selected locations from Separation Canyon to Boulder Dam.

Again, it was not possible to repeat collections to any great extent, but accurate information on distribution was obtained. Chiefly phanerogamic flora was studied, and herbarium specimens were collected on both of these expeditions. A complete report, giving an account of the history, geology, and topography of the Colorado River region, and including a list of collected plants, will be published in the near future.

The expedition of 1938 was financed in part by a Rackham Faculty Research Grant and in part by Edgar J. Marston of Colorado Springs, Colorado.

The extremely high, often sheer walls, and the scouring action of the water increase the problems of migration of plants up the floor of the Canyon. The seeds of cacti are not adapted for wind dispersal, so that distribution must be accounted for in other ways. Some species, especially *Opuntia*

[1] Paper No. 729 from the Department of Botany and the Botanical Gardens, University of Michigan.

[2] Plate 1 is published at the expense of the authors.

*acanthocarpa* and *O. Bigelovii*, grow high on the walls and may be seen in crevices or pockets in the rock to within 100 or 150 feet of the canyon floor. Joints readily break off and probably fall over the rim, and take root if given an opportunity. Run-off water plays a part in bringing the joints lower in the canyon. None of the *Cylindropuntiae* is found on the lower talus or near the water.

The *Echinocerei*, especially *E. Engelmannii*, are found frequently on low ledges and talus, and some may be seen higher on the walls, showing as they always do a preference for limestone. It is noticeable that the caespitose forms have fewer stems than those up on the desert. The extreme heat on the rock walls and a lack of soil may cause sparsity of growth. Many plants have great difficulty in surviving these extreme conditions. This is evidenced by withered and dying cacti on dry ledges. It is interesting to note that the same species may also languish among boulders on deltas and sandy shores. Here is a case where drought vies with flood waters in exterminating plants struggling for existence in a trying situation. Flood waters bring plants down side canyons and farther into the gorge of the Colorado where they lodge between boulders and, often half-buried, take root. *Opuntia basilaris* and others belonging to this series are the only species found near the water's edge. In normal desert habitats they may be found both at high and low altitudes and in the shade of shrubs and trees, as well as among the rocks in the hottest possible places on the desert. The growth in canyons is much less luxuriant, consisting often of only two or three joints. This may be accounted for to some extent by the fact that stems are torn apart by floods or by landslides. These joints take root and barely become established before they are again uprooted.

In contrast, a permanent delta at Nankoweap Rapids (above Bright Angel Creek) supports an abundant and vigorous association of *Opuntia polyacantha*, *O. hystricina*, *O. phaeacantha*, and *Echinocereus Engelmannii* (fig. 1). These, with mesquite and *Acacia*, form a typical desert scrub covering. The many boulders strewn over this area afford protection to plants until they gain sufficient foothold to withstand the wash of flood waters, which in so wide a space cannot be deep and swift.

Certain sections in Havasupai Canyon are particularly favorable to the growth of certain cacti. Supai shale is very productive, and in places *Opuntiae* flourish almost to the exclusion of other plants.

The flora of the canyons studied is typical of the Sonoran zone, as defined by Merriam. An arm of the Upper Sonoran extends north from Lee's Ferry along the Colorado River and for some distance up such tributaries as the San Juan River and Forbidding Canyon. *Atriplex canescens* and *Salix exigua* are typical Upper Sonoran species and cacti associated with them include *Opuntia polyacantha* and *Echinocereus octacanthus*.

At Lee's Ferry the Colorado flows southwest into deeper canyons where the inner and warmer portions of the gorge support a Lower Sonoran flora. Prominent in the vegetation of this area are mesquite, *Acacia*, and creosote bush. Although the last species is recognized as typically Lower Sonoran, it is not common above Lava Falls. Creosote bush (*Larrea*) apparently requires a higher temperature than those species found in the northern reaches of this zone. *Ferocactus acanthodes* (fig. 3) and *Phellosperma tetrancistra* are commonly found in the Lower Sonoran, and the latter occurs northward as far as Rainbow Bridge.

When a southern species is reported over two hundred miles beyond the northern limits of its range, it is possible that a thorough search on the desert would show a more or less continuous distribution.

Conditions seem to be unfavorable for the *Coryphanthae* although members of this genus are abundant in other parts of the Lower Sonoran. Most western species require a relatively high altitude, which may account for their complete absence here.

*Echinocactus polycephalus* Engelm. & Bigelow. Usually in clumps of from several to many rounded heads. Occasional on the Bright Angel shale in Spencer (*4446*) and Separation Canyons. Undoubtedly scattered on walls of Lake Mead to some distance below Pierce Ferry (*4447*) where it is found infrequently. Commonly associated with *Ferocactus acanthodes* but not extending so far north. (Fig. 2.)

*Distribution:* Southern Nevada, California, possibly in extreme southern Utah, western Arizona, northern Sonora and reported from Lower California.

*Echinocereus acifer* (Otto) Lemaire? Caespitose, sometimes solitary, erect, stem bright green, spines brown. There is a possibility that this is a new species. It fits the description of *E. acifer* very well (flowers have not been observed), but is entirely out of the reported range. Fairly common on limestone ledges above the river in Narrow Canyon near the mouth of the Dirty Devil River (*2184*).

*Distribution:* Durango and Coahuila, Mexico; Narrow Canyon, Canyon of the Colorado, San Juan Co., Utah.

*Echinocereus octacanthus* (Mühlenpfordt) Britt. & Rose. Growing on limestone ledges. Mouth of the Dirty Devil River (*2184A*); Surprise Valley; Rainbow Bridge; Vasey's Paradise; above Bright Angel in Grand Canyon; Hermit Creek (*2303*); two miles above Bass Cable (*2317A*); and Elves' Chasm. This represents a considerable extension of range.

*Distribution:* Northwestern Texas; and reported from New Mexico and Utah.

*Echinocereus coccineus* Engelm. This species is often confused with *E.*

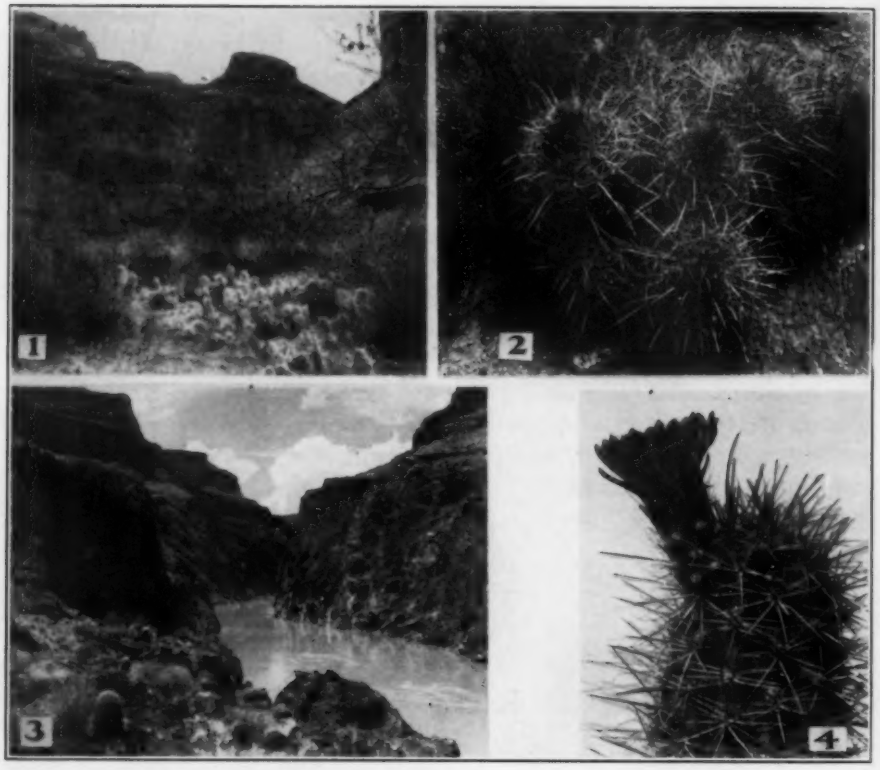



FIG. 1. Well-established association including *Opuntia polyacantha, Echinocereus Engelmannii,* and *Acacia Greggii* growing on the right of the Colorado at Nankoweap Rapids. FIG. 2. *Echinocactus polycephalus;* occasional on steep talus in Lower Grand Canyon. FIG. 3. Habitat of *Ferocactus acanthodes* near Separation Canyon; common throughout the Lower Sonoran within the Canyon of the Colorado and tributaries. FIG. 4. *Echinocereus canyonensis;* occasional at Bass Cable on a limestone outcrop.

*octacanthus* although they are very distinct. Occasional from Narrow Canyon (*2186*) to Lake Mead.

*Distribution:* New Mexico and Arizona to Utah and Colorado.

*Echinocereus Engelmannii* (Parry) Rümpler. More abundant in the Canyon than any other species. It appears well able to adapt itself to difficult situations. Often found clinging to rock ledges hundreds of feet above the floor of the Canyon, probably started from seeds which fell over the rim. This species is also found on steep talus slopes, on limestone ledges above the river, and on boulder-strewn deltas of side canyons. Collections and plants noted at the following locations: Mormon Trail in Glen Canyon; in the canyon of the San Juan River; Rainbow Bridge; Lee's Ferry; President Harding Rapids; Havasupai Canyon (*4334*); Nankoweap Rapids; 205 Mile Can-

yon; Spring Canyon; Diamond Creek; Separation Canyon; Spencer Canyon; Quartermaster Canyon; Boulder Dam. (Map, plate 1.)

*Distribution:* California, Nevada, Arizona, Utah, northern Mexico and Lower California.

*Echinocereus Fendleri* (Engelm.) Rümpler. This is a variable species with spines ranging from ¼ inch to 3 inches in length. Only the long-spined form was found in the Colorado River region. It was first seen in Marble Canyon on an extensive talus at President Harding Rapids (*2281*); Saddle Canyon; Granite Rapids; Elves' Chasm; Havasupai Canyon; usually associated with *E. Engelmannii*, never abundant.

*Distribution:* Texas, Utah, Arizona, Sonora, and Chihuahua.

*Echinocereus mojavensis* (Engelm. & Bigelow) Britt. & Rose. Occasional on talus and limestone or sandstone ledges. Hell Roaring Canyon (*2112*); Dark Canyon; President Harding Rapids; Vasey's Paradise; Spencer Canyon; Separation Canyon.

*Distribution:* Southeastern California, Nevada, Utah, western Arizona.

*Ferocactus acanthodes* (Lemaire) Britt. & Rose. First seen 26½ miles below Lee's Ferry (*2239*); Vasey's Paradise on talus (*2271*); Mile 39 below Lee's Ferry; President Harding Rapids (*2272*); Saddle Canyon; Granite Rapids; Bass Cable; Elves' Chasm; common at Conquistadore Aisle and in Havasupai Canyon; very common at Lava Falls (*2371*); Separation Rapids; Spencer Canyon (*4263A*); Emery Falls (*4254*). These plants are usually found on steep talus slopes, apparently coming into the canyon over the top, judging from their occurrence high on the walls. As the water rose in Lake Mead many of these large plants were submerged, and may be seen floating in the water; others are entirely covered except for an inch or two of bristling spines. (Map, plate 1.)

*Distribution:* Southeastern California, northern Lower California, southern Nevada, southern Utah and northern Arizona.

*Opuntia Whipplei* Engelm. & Bigelow. Never abundant but occurring on upper talus and on ledges. Vasey's Paradise on steep talus near gushing spring; Upset Rapids; two large clumps found on talus near Walthenburg Rapids; Diamond Creek on ledges; 192 Mile Rapids; Havasupai Canyon (*Gard. 17165*[2]). Found down Hualapai Canyon to Havasupai and on to the Colorado.

*Distribution:* Northern New Mexico and Arizona, southwestern Colorado, and southern Utah.

*Opuntia tetracantha* Toumey. Single specimen found growing in sand about two hundred yards up Diamond Creek (*2402*). It is difficult to explain

[2] Here and in following paragraphs this abbreviation refers to the number assigned to a plant in the University of Michigan Botanical Gardens.

its presence here, since the reported range is only in the vicinity of Tucson, Arizona.

*Opuntia acanthocarpa* Engelm. On high ledges and talus from Havasupai Canyon to Boulder Dam. Boulder Dock (*4180*); 217 Mile Canyon; Diamond Creek (*2396*); Quartermaster Canyon (*4277*).

*Distribution:* Southern Utah, Arizona, California. Reported from Sonora.

*Opuntia echinocarpa* Engelm. & Bigelow. Fairly common on steep talus and rock ledges, Spencer Canyon (*4450*).

*Distribution:* Nevada, Utah, Arizona, California, and Lower California.

*Opuntia Bigelovii* Engelm. Found from 205 Mile Canyon (below Lava Falls) to Spencer Canyon. This stretch is considerably south of any other part of the river. 205 Mile Canyon (*2388*); Diamond Creek; Quartermaster Canyon; Spencer Canyon.

*Distribution:* Central and southern Arizona and California, northern Lower California and northern Sonora.

*Opuntia molesta* Brandegee. Diamond Creek (*Gard. 16850*). This is an extension of the range, which may be accounted for by the fact that the genus Opuntia is not well understood and that the area is not well collected.

*Distribution:* Heretofore reported only from Lower California.

Members of the series Basilares occur at intervals from Cataract Canyon just below the confluence of the Green and the Colorado Rivers to Boulder Dam. Most of those in the upper Canyon are small and variable. Their existence seems to be precarious since they are usually found half-buried in sand or lodged between boulders. Occasional plants may be found at the base of the steep talus a short distance above the river.

*Opuntia basilaris* Engelm. & Bigelow. Usually rare, and high up on ledges. Mile 26½ in Marble Canyon; abundant near Vasey's Paradise; Mile 39 below Lee's Ferry; Diamond Creek; Spencer Canyon (*4440*); Separation Rapids; Boulder Dam.

*Distribution:* Northern Sonora, western Arizona, southern California, Nevada, and Utah.

*Opuntia basilaris* Engelm. var. This plant differs from *O. basilaris* in having smaller, narrow joints branching from the base, and extremely small areoles. It answers the description of the horticultural variety *nana*, as given by Borg. Spencer Canyon (*4248*).

*Opuntia brachyclada* Griffiths. Small and caespitose, this species does not produce the broad clumps in the canyon which it does in southern Utah. Mile Rapids, Cataract Canyon (*2111*); and Forbidding Canyon (*2404*), Utah.

*Distribution:* Southern Utah, and Mescal Canyon, San Gabriel Mts., California.

*Opuntia aurea* Baxter. Although this is considerably out of the range and habitat as described by Baxter, the plant appears to belong here. Indian Gardens, Grand Canyon, Arizona (*4165*).

*Distribution:* Both sides of the Arizona-Utah line near the Kaibab Indian Reservation and west to the lower slopes of the Sierras, near Bishop, California; south to near Silver Lake, San Bernardino Co., California. Reported also from Cane Beds, Pipe Springs; between Zion Park and Mt. Carmel; and south on the road to Kanab, Utah.

*Opuntia Vaseyi* (Coulter) Britt. & Rose. Several spreading plants growing on a limestone outcrop two miles above Bass Cable (*2318*). This is another species with a peculiar distribution. Hitherto it has been reported only from San Bernardino, San Diego and Orange Counties, California.

*Opuntia phaeacantha* Engelm. Variable species found occasionally in Marble Canyon and Grand Canyon. Vasey's Paradise on dry talus near gushing spring (*Gard. 16871*); Nankoweap Rapids (*2273*); President Harding Rapids; and Indian Gardens on the Bright Angel Trail (*4167*).

*Distribution:* West Texas to New Mexico, Arizona and northern Mexico.

*Opuntia Engelmannii* Salm-Dyck. A large species with a more southern range than many of this genus; found usually in undisturbed areas where typical desert vegetation is well established. Two miles above Bass Cable (*2316*); listed at President Harding Rapids; Kwagunt Rapids; below Saddle Canyon; very abundant at Granite Rapids; Elves' Chasm; Havasupai Canyon.

*Distribution:* Texas, New Mexico, Arizona, Durango, Chihuahua, and Sonora.

*Opuntia mojavensis* Engelm. Rather common in Havasupai Canyon on top of the red wall limestone below the Bright Angel Shale; common on the flat above Bridal Veil Falls (*4438; Gard. 17169*). Hitherto reported from southern California, southern Utah, and western Arizona.

*Opuntia laevis* Coulter. Below Granite Rapids (left bank) among boulders (*Gard. 16851*).

*Distribution:* Southern Arizona, abundant in the mountains about Tucson.

*Opuntia chlorotica* Engelm. & Bigelow. Occasional near Lee's Ferry (*4335*); Nankoweap Rapids; two miles above Bass Cable (*2316*); Havasupai Canyon (*4441*).

*Distribution:* Both sides of the Colorado from the San Francisco Mts. to the headwaters of the Williams River and to Mojave Creek, Arizona; southern Utah, New Mexico to Nevada, California, Lower California, and Sonora.

*Opuntia erinacea* Engelm. An attractive species conspicuous for long, white, often hair-like spines; found commonly from lower Glen Canyon to

upper Lake Mead. Forbidding Canyon (*2404*); Nankoweap Rapids on the very dry areas away from the river; Havasupai Canyon (*4434*) on Supai formation above Bridal Veil Falls; listed at Bass Cable and Separation Rapids.

*Distribution:* Northwestern Arizona, southern Utah, southwestern Colorado, southern Nevada, and eastern California.

*Opuntia hystricina* Engelm. & Bigelow. A doubtful specimen was found at Hell Roaring Canyon, growing with *O. rhodantha*. These two species somewhat resemble each other and the collected specimen may be an unusual form of *O. rhodantha* (*2081*); Forbidding Canyon on sand "hills" and ledges (*2401*). A robust form was found at Lee's Ferry on a rocky hillside near the Colorado. The joints were ¾ inch thick with reflexed flattened strong spines (*4333*). E. W. Nelson first reported a form collected here in 1909. Also seen at Saddle Canyon; Nankoweap Rapids; and Havasupai Canyon.

*Distribution:* New Mexico, Arizona, and Nevada.

*Opuntia rhodantha* Schumann. This species is fairly common along the Green River and the upper Colorado. Hell Roaring Canyon (*2078, 2079, 2082, 2100*); Lee's Ferry, on rocky hillside near the Colorado (*4300*).

*Distribution:* Northern Arizona, Utah, Colorado, western Nebraska.

*Opuntia polyacantha* Haworth. A widespread species of varying forms. Near Greenriver, Utah (*1990, 2016,* the latter a red-flowered form); Mile 84 on the Green River; abundant at Hell Roaring Canyon; Cataract Canyon; Forbidding Canyon; Lee's Ferry, Tanner Rapids; in Grand Canyon at President Harding, Nankoweap, and Kwagunt Rapids; Hermit Creek; Granite Rapids. (Map, plate 1.)

*Distribution:* North Dakota to Nebraska, northwest Oklahoma, west Texas, Arizona, Utah, Washington, and Alberta.

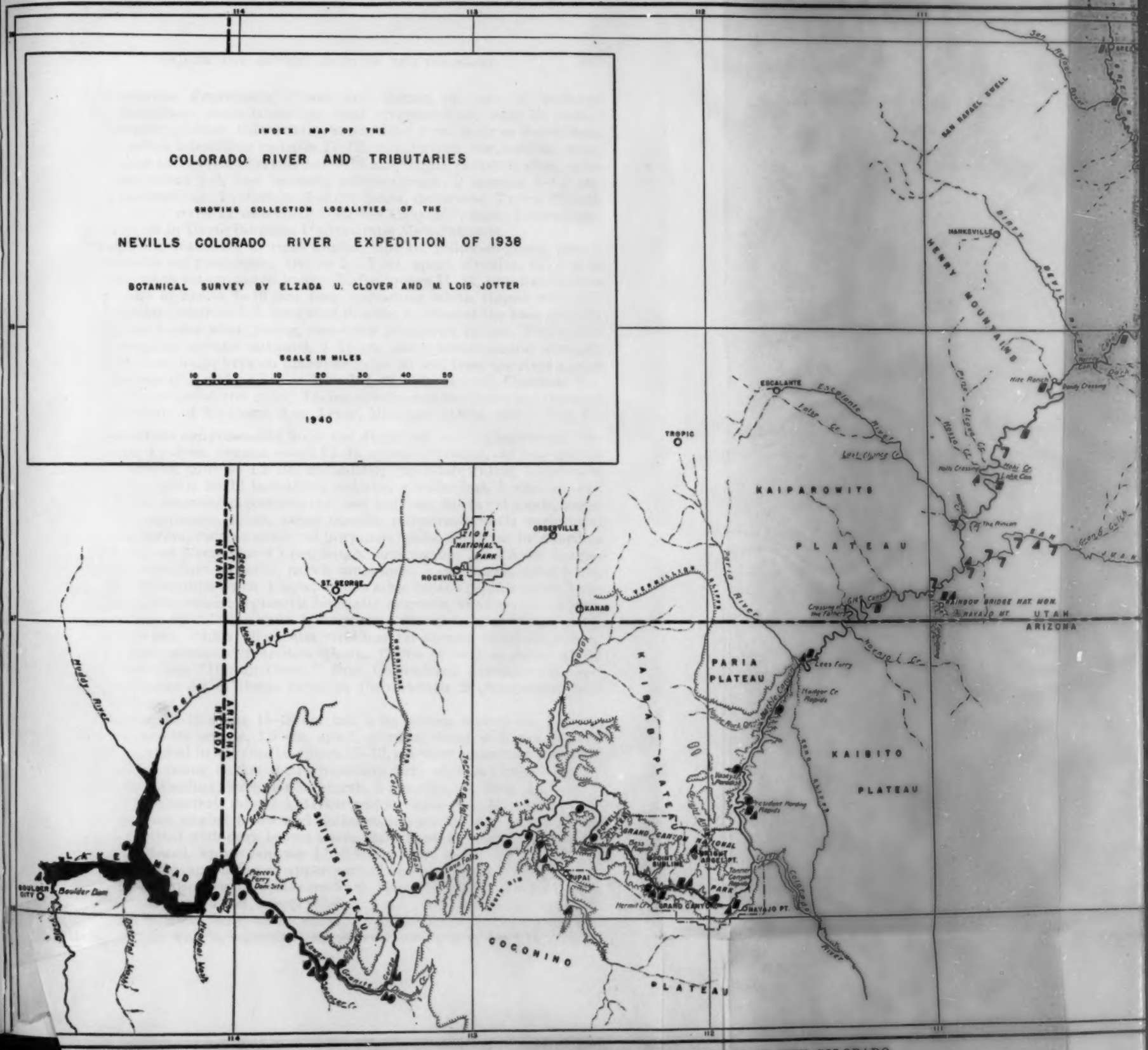
*Phellosperma tetrancistra* (Engelm.) Britt. & Rose. This genus appears first at the mouth of Forbidding Canyon, near Rainbow Bridge; Mile 26½ in Marble Canyon (*2240*); Vasey's Paradise; Saddle Canyon; Granite Rapids; Bass Cable; Elves' Chasm; Havasupai Canyon (*4442*); Diamond Creek; Separation Canyon (*4247*); Spencer Canyon; Emery Falls (*4287*).

*Distribution:* Western Arizona, southeastern California, southern Utah, southern Nevada and probably northern Lower California.

*Sclerocactus Whipplei* (Engelm. & Bigelow) Britt. & Rose. On the Green River at Mile 84; Hell Roaring Canyon.

*Distribution:* Southeastern Utah and western Colorado.

*Sclerocactus polyancistrus* (Engelm.) Britt. & Rose. Below Mile Rapid (*2113*) on hot canyon wall. The fruits of this specimen were pink instead of magenta as described.


INDEX MAP OF THE
COLORADO RIVER AND TRIBUTARIES
SHOWING COLLECTION LOCALITIES OF THE
NEVILLS COLORADO RIVER EXPEDITION OF 1938
BOTANICAL SURVEY BY ELZADA U. CLOVER AND M. LOIS JOTTER
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1940
KAIPAROWITS PLATEAU
PARIA PLATEAU
KAIBAB PLATEAU
KAIBITO PLATEAU
SHIVWITS PLATEAU
COCONINO PLATEAU
HENRY MOUNTAINS
ESCALANTE
TROPIC
KANAB
ORDERVILLE
ROCKVILLE
ST. GEORGE
HANKSVILLE
LAKE MEAD
BOULDER CITY
Boulder Dam
UTAH
ARIZONA
NEVADA
GRAND CANYON NATIONAL PARK
Rainbow Bridge Nat. Mon.
Navajo Mt.
Lees Ferry
Navajo Br.
Glen Canyon
Escalante River
Paria River
Virgin River
Muddy River
Colorado River

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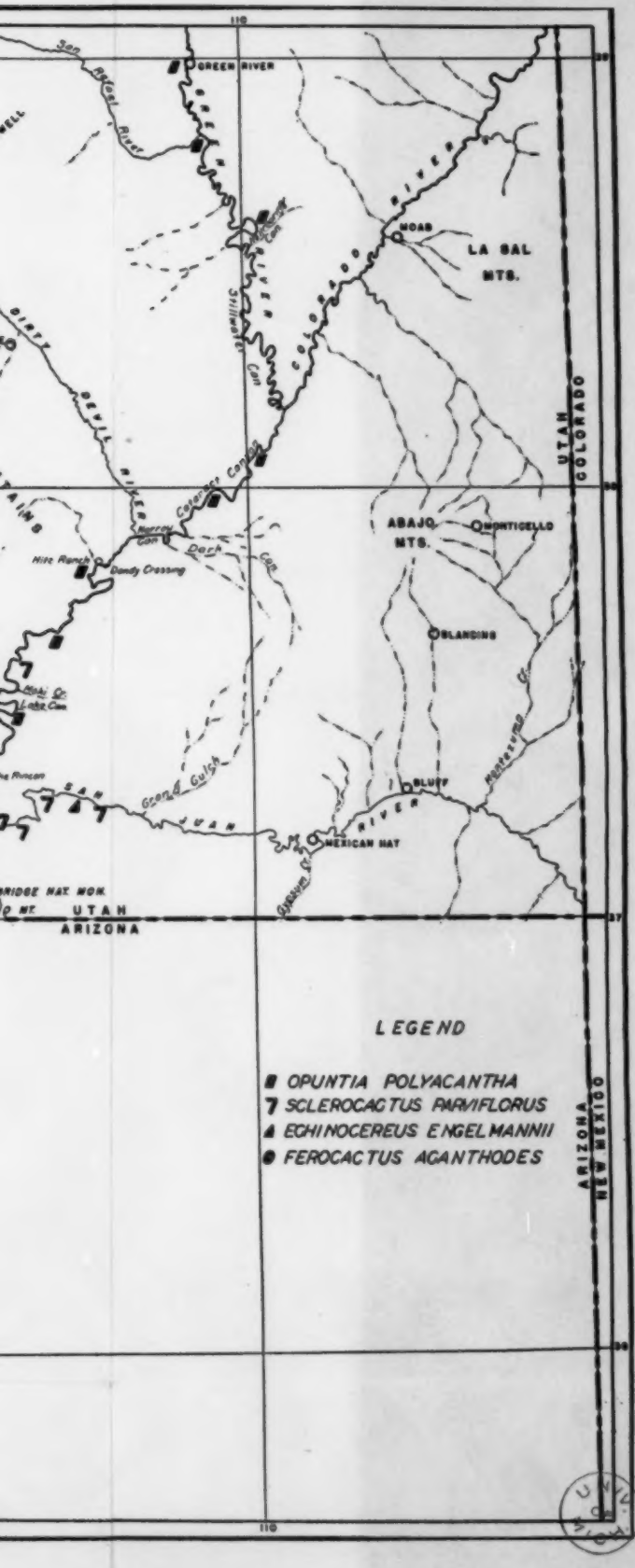
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GREEN RIVER
GREEN RIVER
COLORADO RIVER
MOAB
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ABAJO MTS.
MONTICELLO
BLANDING
BLUFF
MEXICAN HAT
SAN JUAN RIVER
UTAH
ARIZONA
ARIZONA
NEW MEXICO
LEGEND
OPUNTIA POLYACANTHA
SCLEROCACTUS PARVIFLORUS
ECHINOCEREUS ENGELMANNII
FEROCACTUS ACANTHODES

**Echinocereus decumbens** Clover and Jotter, sp. nov. Cylindricus simplex, decumbens, ramis lateralibus recte divergentibus; costis 10, prominentibus, luteo-viridibus, subcrenatis; areoleis 1–1.3 cm. inter se distantibus, rotundis; spinis lateralibus radiatis 11–12, acicularibus, inequalibus, superioribus quam inferioribus brevioribus 5–10 mm. longis, porrectis albis, apice fuscis; centralibus 2–3, basi bulbosis, subcompressis; 2 summis 1–1.5 cm. longis, ascendentibus; 1 inferiore, 2–3 cm. longa, decurvata. TYPUS (*Clover et Jotter 2212*) vivus ex loco dicto "Marble Canyon"; flum. Coloradensi, conservatus est in Horto Botanico Universitatis Michiganensis.

Decumbent, branching at right angles; ribs 10, yellowish-green, prominent, tubercles not prominent; areoles 1–1.3 cm. apart, circular, filled with tawny wool when young, naked in age. Radial spines 11–12, unequal, shorter in upper part of areole, 5–10 mm. long, spreading, white, tipped with red-brown, acicular; centrals 2–3, somewhat flexible, bulbous at the base, slightly flattened, red-brown when young, rose-color to whitish in age. Two upper centrals pointing up and outward, 1–1½ cm. long, lower central strongly deflexed, 2–3 cm. long; TYPE on limestone ledge 30 feet from the river's edge and at the base of a rocky talus at Mile 16½, Marble Canyon, Coconino Co., Arizona (*Clover and Jotter 2212*). Living specimen at the Botanical Gardens of the University of Michigan, Ann Arbor, Michigan (*Gard. 16870*. Fig. 7).

**Echinocereus canyonensis** Clover and Jotter, sp. nov. Caespitosus, 15–20 cm. altus, 4.5–5 cm. crassus, costis 12–13, cyanei-viridibus, obtusis; areolis rotundis, remotis, inter se 1.5 cm. distantibus, juventate fulvis, tomentosis, aetate nudis; spinis 10–12 lateralibus radiatis, acicularibus, 5 mm.–2.5 cm. longis, rigidis; centralibus plerumque 4, basi bulbosis, fuscis vel nigris, angularibus vel compressis, luteis, saepe maculis purpureo-bruneis variegatis; spinis vetustioribus cinereo-roseis vel purpureo-roseis, porrectis, inferioribus uncinatis, 2.5 vel plerumque 4.5 cm. longis, superioribus 1.5–2.5 cm. longis; floribus in summitate solitariis, parvis sanguineis, 5 cm. longis, 4.3–4.5 cm. crassis; bracteis rubris 2 mm. longis; areolis albis, lanatis; spinis quam 1 cm. brevioribus, apice roseis; segmentis biseriatis, angustis, viridescentibus basi, 5 mm. crassis, apiculatis; stylo viridescenti, stigmatis lobis viridibus 8, plerumque 6 mm. longis; filamentis viridibus vel sursum rubri-purpureis; antheris rubri-purpureis; fructibus ignotis. TYPUS (*Clover et Jotter 2317*) vivus ex loco dicto "Hermit Creek," flum. Coloradensi, Coconino Co., Arizona, conservatus est in Horto Botanico Universitatis Michiganensis, Ann Arbor, Michigan.

Caespitose, 12–15 stems, 15–20 cm. tall, 5 cm. across, blue-green, ribs 12–13, obtuse; areoles remote, 1.5 cm. apart, circular, filled with tawny wool when young, naked in age; radial spines 10–12, acicular, somewhat flattened, yellowish when young, darker at tips, becoming dirty white to purplish in age, pectinate to spreading, irregular in length, 5 mm.–2.5 cm. long (commonly 1.5 cm.), rigid; centrals mostly 4, darker and stronger than the radials, bulbose at the base, angled below and flattened toward the tip, young spines yellowish, mottled with dark brown above, light beneath, porrect, lower one somewhat deflexed, upper centrals 1.5–2.5 cm. long, lower one 2.5–4.5 cm. long. Flower buds appearing at upper part of areole, flowering February 22 and remaining open five days; flowers 5 cm. long, 4.3–4.5 cm. across, areoles on ovary about 1 cm. apart, bracts reddish, 2 mm. long, areoles white wooly, spines short to 1 cm. long, white, sometimes pink-tipped; petal-like part of perianth in two whorls, segments very narrow, greenish at the base, scarlet

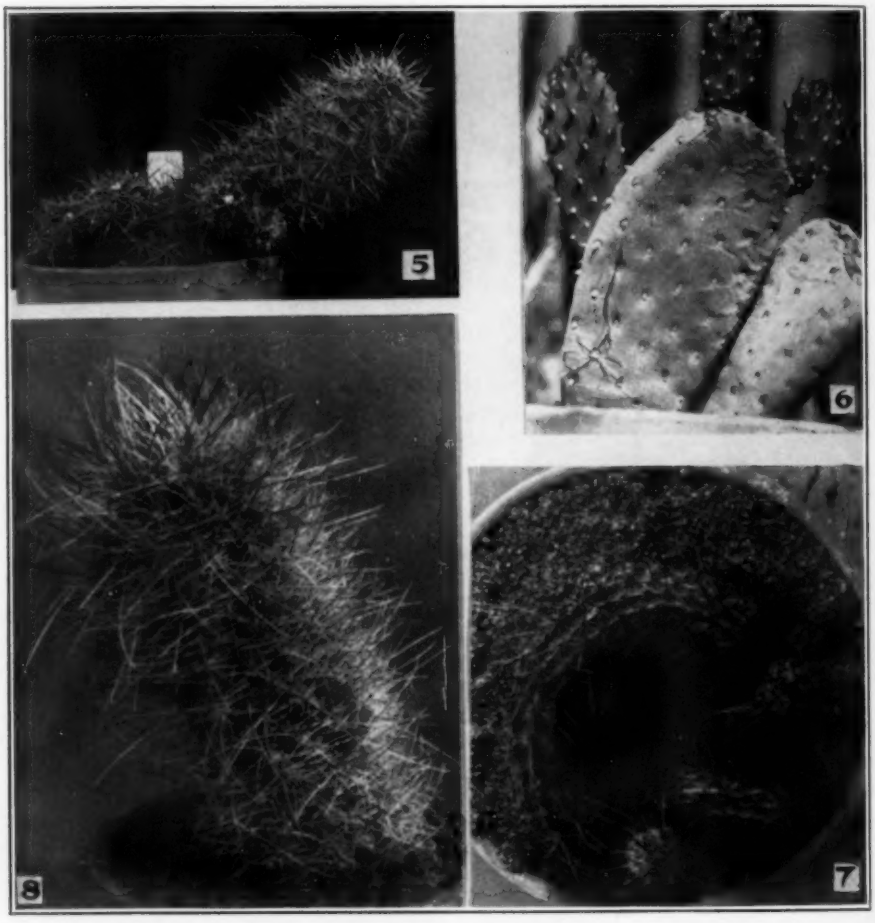


FIG. 5. *Echinocereus canyonensis.* FIG. 6. *Opuntia longiareolata;* rare on steep talus, Granite Rapids. FIG. 7. *Echinocereus decumbens;* single specimen, very different in habit from any other *Echinocereus* in the Canyon. FIG. 8. *Sclerocactus parviflorus;* abundant in lower San Juan Canyon and in restricted areas in Glen Canyon.

toward the tip, apiculate; style greenish, stigma lobes 8, bright green, 6 mm. long; stamens reaching to base of stigma lobes, filaments green below, reddish-purple above, anthers reddish-purple. TYPE (*Clover and Jotter 2317*) collected in a sandy pocket on a steep limestone outcrop 100 yards from the river, Bass Cable below Hermit Creek Rapids, Grand Canyon, Coconino Co., Arizona. Locally abundant. Living specimen in the Botanical Gardens (*Gard. 16846*), University of Michigan, Ann Arbor, Michigan. (Figs. 4, 5.)

**Opuntia longiareolata** Clover and Jotter, sp. nov. Humilis, articulis spathulatis, glaucescentibus, pubescentibus, adscendentibus e basi proliferis; foliis subulatis minutis, depressis, viridi-rubellis, curvatis; areolis subconfertis, depressis albo-tomentosis, setis numerosis brevibus, roseis vel stramineis, lana circumdatis, demum nudatis; floribus ignotis. TYPUS (*Clover et*

*Jotter 2302*) vivus ex loco "Granite Rapids," flum. Coloradensi, Coconino Co., Arizona, dicto conservatus est in Horto Botanico (*Gard. 16852*) Universitatis Michiganensis.

Plant belonging to the Series Basilares; joints few, branching from the base and also from top and sides of joints; joints tomentose, elongated, 10–12 cm. long and 4.5 cm. across, spathulate; areoles 8–10 mm. apart, circular on very young joints, these filled with white wool entirely surrounding the pinkish to straw-colored glochids which are early-deciduous, areoles very soon becoming much elongated, 3 mm. long and 1 mm. wide; leaves 4 mm. long, green with burgundy tip, curved, awl-shaped; old joints becoming pale yellow-green in contrast to the deep blue-green of young ones. TYPE (*Clover and Jotter 2302*) growing at base of steep talus, near water's edge, Granite Rapids (*Gard. 16852*), Grand Canyon, Coconino Co., Arizona. (Fig. 6.)

**Sclerocactus parviflorus** Clover and Jotter, sp. nov. Simplex cylindricus erectus, vel curvatus, 1–4.5 dm. altus, 6–9 cm. crassus; areolis orbiculatis; costis 13, tuberculis prominentibus; spinis 14–15 lateralibus, radiatis, compressis, superioribus quam inferioribus brevioribus, 3.4 cm. longis, apice adustis; spinis centralibus 3 angulatis, summa singula triquetra, alba apice adusta, plerumque curvata, flexuosa, 5–6 cm. longa, ascendenti; inferioribus triquetris vel quadrangulatis, purpureofuscis, 6–7.5 cm. longis, uncinatis, decurvatis; lateralibus 2 purpureo-fuscis, 5.5–6 cm. longis, porrectis; floribus in axillis summis solitariis, parvis, 2.5 cm. longis, 2 cm. crassis, exterioribus in media parte atropurpureis, marginibus et apice scariosis; interioribus purpureis, segmentis crassis, 2-seriatis, apice acuminatis, erosis; stylo purpureorubro, stigmatis lobis 10, filamentis viridibus, numerosis, delicatis, antheris luteiaurantiacis, quam stylo brevioribus; fructibus siccis. TYPUS (*Clover et Jotter 2398*) vivus ex "Forbidding Canyon," flum. Coloradensi, Coconino Co., Arizona, dicto conservatus est in Horto Botanico (*Gard. 16845*) Universitatis Michiganensis, Ann Arbor, Michigan.

Plants solitary, oblong, tapering slightly toward the tip, 1–4.5 dm. high; ribs 13, strongly tuberculate; areoles 1.5 cm. apart, sub-circular, plant entirely covered by a dense mass of long, curved and twisting spines; radials 14–15, white with dark tips, much flattened, heavier and longer, 3.4 cm. in the lower part of the areole, central spines 4, the upper one white to horn-colored, tipped with red-brown, 3 angled, flexible, 5–6 cm. long, pointing upward, the lower central 3–4 angled, 6–7.5 cm. long, pointing downward, the two lateral centrals 5.5–6 cm. long, slightly ascending; all centrals except uppermost reddish and curved or hooked. Flowered April 17, remaining open for three days; bud conic, brownish; flower 2.5 cm. long, 2 cm. across; outer perianth segments phlox-purple, in 2 whorls of 8 segments each; these broad with erose margins, acuminate, tube scarcely present; style purplish, stout, longer than the stamens, stigma lobes 10, flattened below, curving outward, 1.5–2 mm. long, amaranth purple; stamens bright orange-yellow, filaments green, very delicate; fruit unknown. TYPE collected at the mouth of Forbidding Canyon in Glen Canyon above Lee's Ferry, Canyon of the Colorado. Abundant 20 miles above Moki Creek, and fairly common at intervals along the lower San Juan River (plate 1). Living specimen (*Clover and Jotter 2398*) at the Botanical Gardens (*Gard. 16844*), University of Michigan, Ann Arbor, Michigan. (Fig. 8.)

UNIVERSITY OF MICHIGAN
ANN ARBOR, MICHIGAN

# INDEX TO AMERICAN BOTANICAL LITERATURE

The aim of this Index is to include all current botanical literature written by Americans, published in America, or based upon American material; the word America being used in the broadest sense.

Papers that relate exclusively to bacteriology, forestry, agriculture, horticulture, manufactured products of vegetable origin, or laboratory methods are not included. If users of the Index will call the attention of the editor to errors or omissions, their kindness will be appreciated.

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